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Typeset and
Printed in Canada by: The Runge Press Limited, Ottawa

Composé et
Imprimé au Canada par: The Runge Press Limited, Ottawa

Design: Ludvic Saleh & Associates, Ottawa

Conception graphique: Ludvic Saleh & Associates, Ottawa

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937 et *Journal de l'office des recherches sur les pêcheries du Canada* 1938–1979.

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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 49, No. 8, August 1992 **Volume 49, nº 8, août 1992**

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Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, Chemical Abstracts, Current Awareness in Biological Sciences, Current Contents, FAO Freshwater and Aquaculture Contents Tables, FAO Marine Science Contents Tables, Oceanic Abstracts, and Science Citation Index

Biomass Spectra of Aquatic Ecosystems in Relation to Fisheries Yield

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Boudreau, P. R., and L. M. Dickie. 1992. Biomass spectra of aquatic ecosystems in relation to fisheries yield. Can. J. Fish. Aquat. Sci. 49: 1528–1538.

The biomass density of aquatic ecosystems can be expressed as an allometric function of organism body size. The log–log plot of this relation, termed the biomass spectrum, is used to compare aquatic ecosystems in various parts of the world. We develop a standardized presentation for several example environments where detailed data on biomass density by body size in the trophic positions, phytoplankton, zooplankton, benthos, and fish, make it possible to establish overall or primary spectral slopes. The basic methodology is adapted for application to other ecosystems where less detailed data are available. Spectra from all the different environments exhibit a uniform low slope, but with different intercepts that appear to reflect ecosystem differences in nutrient circulation and availability. Detail on the secondary structuring at various positions in the trophic system appears to provide information useful for distinguishing between long-term changes in productivity and short-term perturbations in biomass or abundance.

La densité de la biomasse présente dans un écosystème aquatique peut être exprimée sous forme de fonction allométrique de la taille des organismes. On utilise le graphe log–log de cette relation, appelée spectre de biomasse, pour comparer les écosystèmes aquatiques de diverses parties du globe. On élabore aussi une présentation normalisée de plusieurs types d'environnements pour lesquels des données détaillées sur la densité de la biomasse en fonction de la taille des organismes au niveau trophique, zooplancton, phytoplancton, benthos et poisson, permettent d'établir la pente globale ou primaire du spectre. On adapte les méthodes fondamentales pour qu'elles s'appliquent à d'autres écosystèmes pour lesquels des données détaillées ne sont pas disponibles. Les spectres obtenus de tous les différents environnements montrent une faible pente uniforme, mais les différents interceptes semblent réfléter des différences dans le cycle et la disponibilité des aliments au niveau des écosystèmes. Des détails sur la structure secondaire à diverses positions du système trophique semblent fournir de l'information utile dans la séparation des variations à long terme de la productivité et des perturbations à court terme de la biomasse ou de l'abondance.

Received July 15, 1991
Accepted February 7, 1992
(JB145)

Reçu le 15 juillet 1991
Accepté le 7 février 1992

Predicting biological production in communities from energy flux through its trophic components has been a prime focus of ecology. Attempts to use Lindeman's (1942) original formulation, specifying predator–prey trophic linkages between species grouped into ''levels,'' have demonstrated the difficulties of arriving at models that are both sufficiently realistic and sufficiently general (Lane and Collins 1985). The practical failure of the trophic level calculations (Mann et al. 1989) in the past is due partly to the complexity of the systems modelled and partly to continued uncertainty about the time and space scales on which it is possible to distinguish changes in density from changes in abundance (Boudreau and Dickie 1989).

By contrast, measures of biological production (P), respiration (R), and specific production (P/B) per unit area occupied by communities show stable patterns of change in relation to individual organism body size (Humphreys 1979; Banse and Mosher 1980; Dickie et al. 1987). These are reflected in stable patterns of biomass density (B) with body size that appear general enough to imply common regulatory mechanisms (Boudreau et al. 1991; Thiebaux and Dickie 1992; M. L. Thiebaux and L. M. Dickie, unpubl. data). Characterization in the spectrum should therefore provide an alternative to species typologies and trophic networks for understanding ecosystem production dynamics and predicting yield. We test this possibility using data from several freshwater and marine aquatic ecosystems in various parts of the world.

Standardization of the Spectrum

It is necessary to standardize data from different sources so that they exhibit a comparable overall level, slope, and internal structure in relation to body size classes, independent of the various sampling methodologies and of the range of both body size and biomass concentration. The original descriptions by Sheldon et al. (1972, 1973) plotted particle concentration against either an octave or base 10 logarithmic scaling of the body size classes in ''equivalent spherical diameter.'' The spectra had generally low slopes, showing that biomass concentration decreases at a low, more or less constant rate with body size. However, the spectra contained prominent internal structure, and the variability made it difficult to establish the significance of the shapes or the generality of slopes and positions over area and time.

Sprules et al. (1983, 1991) and Schwinghamer (1981, 1983, 1985) used logarithmic ordinal scales of biomass to illustrate the patterns of variability over broad size groups and environments. Schwinghamer (1985), Witek (1986), and Witek and Krajewska-Soltys (1989) showed that the internal, secondary structuring represented by the peaks and troughs of biomass

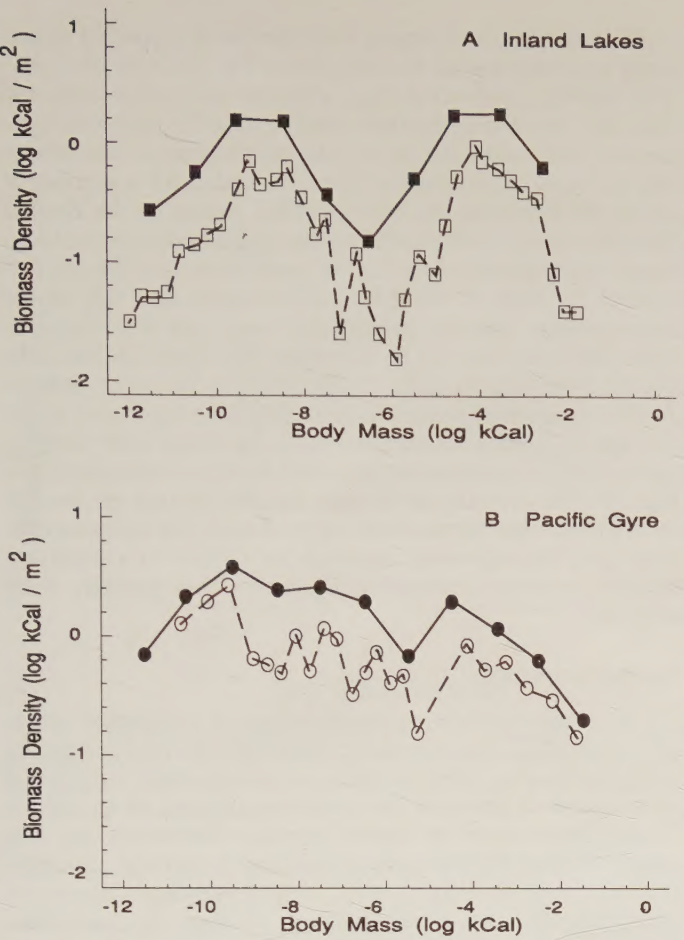
A Inland Lakes
Biomass Density (log kCal / m^2)
Body Mass (log kCal)
B Pacific Gyre
Biomass Density (log kCal / m^2)
Body Mass (log kCal)

FIG. 1. Biomass spectra for inland lakes and North Pacific Central Gyre data plotted unstandardized (broken lines) and standardized (solid lines).

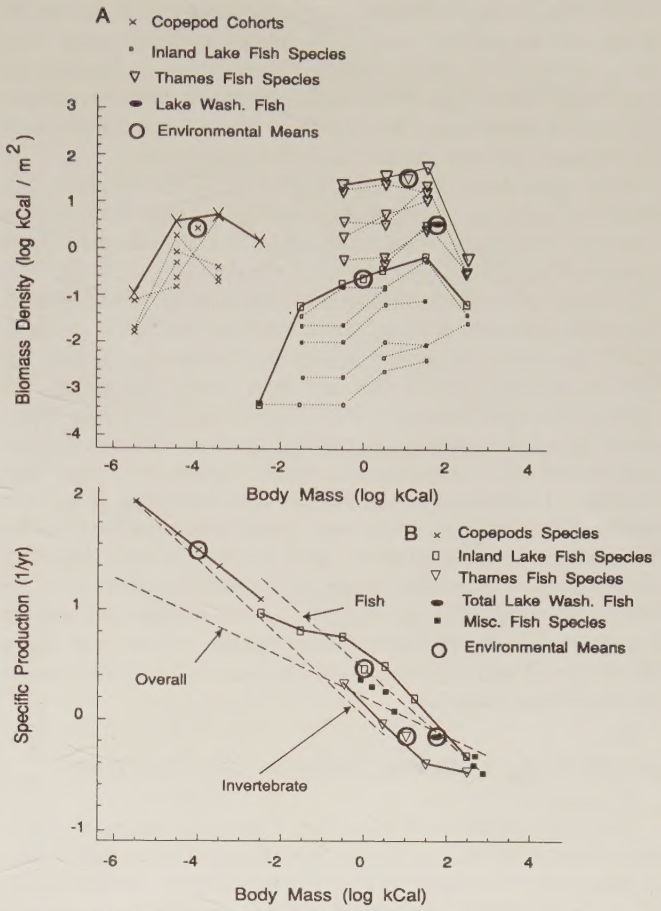

FIG. 2. (A) Biomass and (B) specific production spectra for fish and invertebrate species and species groups from various environments along with corresponding environmental means. The broken lines in Fig. 2B are from Boudreau and Dickie (1989): solid lines represent summations over species.

density was not a result of sampling methodology but a recurrent feature of biomass distributions. Schwinghamer (1985) found that the secondary structuring was consistent within environments and related to the physical composition of the substrate. Boudreau et al. (1991) pointed out that the structuring is directly related to the trophodynamics of the ecosystem.

The alternative representation introduced by Platt and Denman (1978) normalized biomass density by the width of the body size classes. The method is particularly useful for mathematical treatment of the spectrum (Thiebaux and Dickie 1992) and highlights the allometric basis for the density spectrum. It also reduces the apparent effects of variation due to the secondary structure of the spectrum, drawing attention to the overall slopes and intercepts. Rodriguez and Mullin (1986) adopted the normalized biomass spectrum to describe changes in the pelagic zone of the North Pacific Central Gyre.

Dickie et al. (1987) identified two scales of variation of the underlying production processes in relation to body size, which Boudreau et al. (1991) pointed out are related directly to the biomass spectrum. The overall or primary spectrum of body sizes from phytoplankton to fish has a low, uniform, negative slope reflecting the underlying allometric relation between individual metabolism and body size. The secondary scaling, originally observed as a more rapid change in specific production with body size when organisms are separated into quasi-taxonomic groupings such as all fish or all large invertebrates, termed trophic positions (Levine 1980), represents body size groups that have a common production efficiency (P/R) (Humphreys 1979) and appears as a series of distinct domes in the biomass density spectrum. Boudreau and Dickie (1989) concluded that both scalings need to be taken into account to understand and predict community biomass structure and production.

In the standardization adopted here, the basic allometric form of the spectrum is used without normalization. This allows appreciation of the relative importance of the primary and secondary scales of variation in the data for individual trophic positions, on which we rely for construction of the overall spectrum.

In what follows, we use volumetric or mass measures, expressed in the equivalent energy units, kilocalories (kcal) (1 kcal = 1.1868 kJ), for both body size and biomass, hence characterizing the spectrum directly in energy units. Representation of biomass density by body mass classes in unit log base 10 steps of kcal generally yields four or more points within each trophic position, sufficient to define peaks in the spectrum. Examples are given in Fig. 1. This representation allows identification of both the primary and secondary structure of the spectrum. Biomass density data from various sources (see Appendix) have thus been recalculated and regrouped into identical $\log_{10}$ kcal body mass classes. These ordinal measures can be converted into a normalized spectrum by dividing each biomass class by the corresponding average body mass.

Biomass density for the aquatic community can be compiled on either a unit volume or unit area basis. Both have been published, but there are a number of reasons for favouring the unit area alternative. Biomass density is related to energy flux which

begins with surface radiation, and organisms which take up the energy are frequently found to be related to the physical interfaces, sea surface, pelagic zones, thermoclines, or benthic surfaces near which they live, while exhibiting heterogeneity in the vertical dimension. We represent biomass concentration as log biomass per unit surface area of environment.

In the partial spectra given in Fig. 1, we show the previously published and our standardized versions of data from several studies. The inland lakes data (Fig. 1A) are all taken from Sprules et al. (1983). The Pacific Gyre data (Fig. 1B) are from Rodriguez and Mullin (1986) and Beers et al. (1975, 1982). Our compilation over wider size class bins than in the originals results in a differential increase of the biomass in the two figures, resulting in levels that are more nearly the same. The aggregation also results in some smoothing, clarifying representation of the two main trophic positions involved, phytoplankton and zooplankton, while suppressing the finer structure. In the case of the Pacific Gyre spectrum (Fig. 1B), where different size classes were used to summarize data for the positions, standardization decreases the overall slope and gives rise to a somewhat flatter spectrum. Comparison of the two standardized spectra suggests that the marine data contain a greater density of large phytoplankton in the size range between -8 and -6 $\log_{10}$ kcal than seems to be the case in the freshwater samples.

Determining Sampling Scales for the Spectrum

Biomass spectra such as in Fig. 1 relate densities of planktonic organisms over several orders of magnitude in body sizes, to which we wish to add data on benthos and fish. Such extremes of size and turnover time pose difficult questions of how best to sum biomass densities over species, times, and areas. We discuss solutions in connection with specific examples.

Aggregating over Species

Figure 2A presents data for six fish species sampled following the poisoning and clearance of a small freshwater lake (Chadwick 1976), data for five species of fish sampled from the River Thames over a period of years (Mann 1965; Mathews 1971), and data for six copepod zooplankton species collected by various workers using a variety of nets at various times on the Scotian Shelf (McLaren et al. 1989). The freshwater lake data give biomass, production, and average size for each age class of each fish species in the total population. The Thames fish data and Scotian Shelf plankton data are annual averages for cohorts of various species present on the different sampling occasions.

Differences in biomass level among individual species within each of the three environments reflect expected variation in abundance or rarity of species. Their sums will be influenced by the dominant species of the environment; for example, the invertebrate biomass maximum might have occurred at a larger body size if the data set had included the larger zooplanktors such as euphausiids, which were not sampled. Their apparent abundance may also be influenced by the selectivity of the sampling gears. However, the aggregated biomass curves resulting from summation over species with different life histories but similar production efficiencies exhibit a regularity that could not have been anticipated from the individual species data alone. They clearly represent a subsuming of the individual species energetics in the production dynamics of an interactive community.

The rationale for summing over species is supported empirically by comparing the biomass data of Fig. 2A with the equivalent specific production ($\log_{10} P/B$) for each environment in Fig. 2B. The diverse biomass density data for individual species and their sums fall on or close to the general invertebrate and fish specific production lines calculated for a number of species by Boudreau and Dickie (1989), except for the Thames fish data which, while paralleling the invertebrate and fish lines, appear anomalously closer to the invertebrate line. Means calculated for each of the three environments also fall on the corresponding specific production lines, and a comparable aggregate value that can be calculated from fish data for Lake Washington (Eggers et al. 1978) falls into the same pattern. Additional biomass and production data for a number of single fish species from other environments for which total environmental means could not be calculated have also been plotted in Fig. 2B. The diversity of biomass densities among species and environments but the similarity of production per unit area confirms that the organisms involved are subject to a relatively simple, common production dynamics that justifies their addition.

Spatial Averaging

Lake studies can take advantage of natural geographic limits, such as physical lake boundaries or drainage basin contours, to delimit areas over which samples can be reasonably aggregated or averaged to represent an ecosystem (Sprules et al. 1991). Larger, open-ocean or coastal benthic ecosystems are less clearly defined by "objective" criteria. In general, assumptions are made to define marine ecosystem boundaries on the basis of depth strata, current patterns, or other physical or biological limits within which it is reasonable to expect homogeneity if not totality of energetic exchanges between trophic levels.

Temporal Averaging

At temperate latitudes, primary production by the fast-growing phytoplankton is characterized by one or two pulses of production in the spring and fall. The more stable biomass density levels of the larger, slow-growing, multiaged populations of fish are supported by the summation of these pulses of production over their life span. The feasibility of using annual averages to characterize the dynamics has been discussed by Mann (1969), Dickie (1972), Waters (1977), and Banse and Mosher (1980). It is generally concluded that average annual levels of specific production capture the pattern of energy exchange and availability in temperate regions, although it must be recognized that, in general, sampling frequencies need to be set in direct relation to the turnover times of the species (Denman et al. 1989).

To illustrate the extent to which intraannual variability may influence biomass spectra, we calculate standardized spectra for the Georges Bank ecosystem using phytoplankton and zooplankton data for different months of the year (Backus and Bourne 1987). One point is available for each of the trophic positions in each of eight months. The resulting plot in Fig. 3 is a set of lines for a mean-sized phytoplankton and zooplankton organism, from which an annual average can be calculated. The spring phytoplankton bloom is evident in the spectra for March and April, and as the year progresses, the level of phytoplankton density decreases and zooplankton density increases, leading to a positive slope for June. Similar intraannual changes in spectra are evident in McAllister (1969) for the Gulf of Alaska

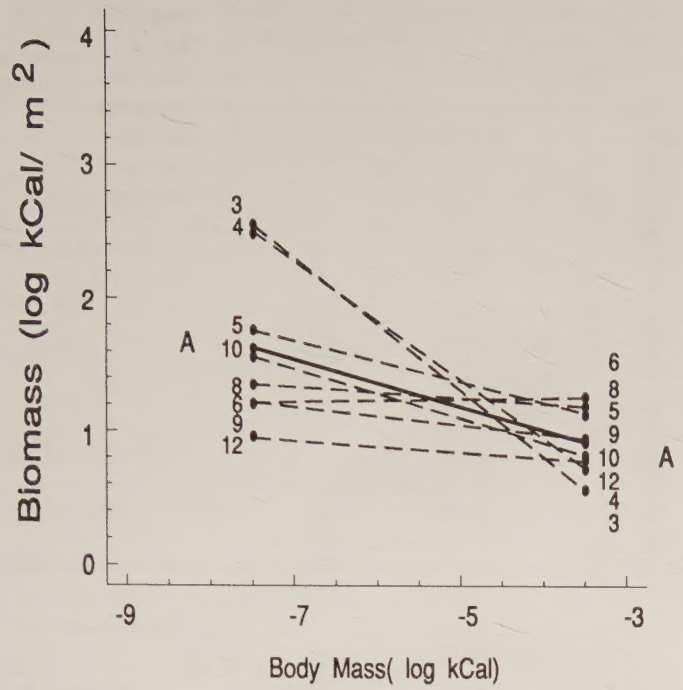

FIG. 3. Standardized biomass spectra for different months of the year for Georges Bank phytoplankton and zooplankton. Numbers represent the month of the year. The highlighted solid line, *A*, is the annual average for the ecosystem.

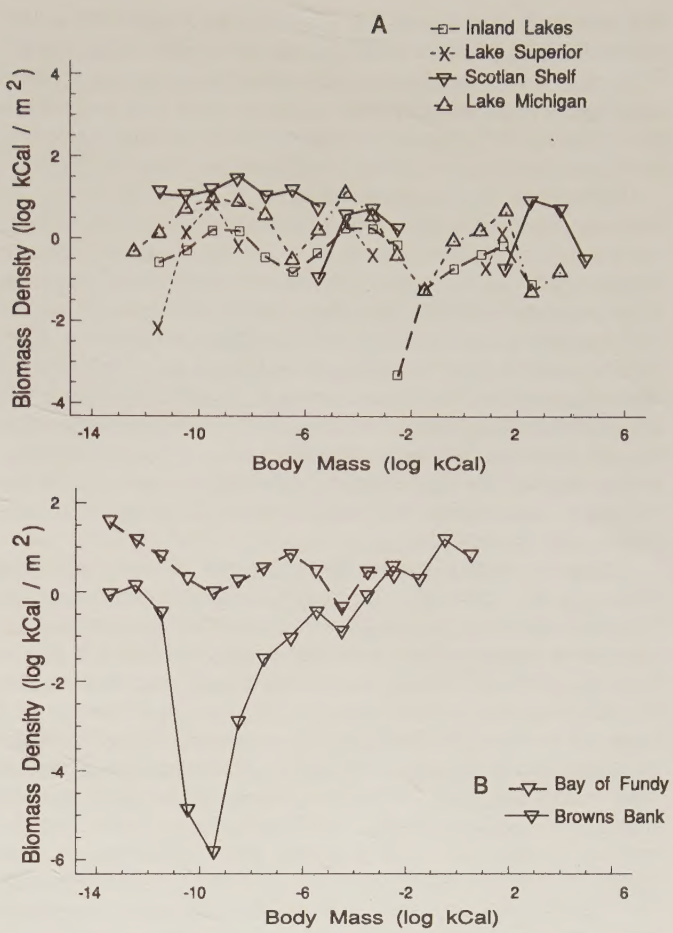

FIG. 4. Standardized biomass spectra for (A) four pelagic and (B) two benthic ecosystems.

and for Lake Michigan (Sprules et al. 1991). In the Georges Bank case, the annual average represents a midpoint in the intraannual variation in slope and level, comparable with annual biomass density spectra for areas with other patterns or timings of the seasonal pulsing. An adequate annual average clearly requires careful scrutiny of the sampling.

Standardized Spectra for Pelagic and Benthic Ecosystems

There seem to be few studies from which it is at present possible to compile a standardized biomass spectrum for the whole range of interacting body sizes. The paper by Sprules et al. (1991) for the pelagic zone of Lake Michigan is an exception. Figure 4A shows standardized spectra for this and three other pelagic environments that have been sufficiently well sampled to allow assembly of detailed data. Figure 4B shows the benthic data available for one of them. These are as follows: (1) Laurentian Shield inland lakes, where the phytoplankton and zooplankton data are the Fig. 1A data from Sprules et al. (1983) and the fish data are from Chadwick's (1976) study (Fig. 2A) of a similar type of lake; (2) Lake Superior, where Sprules and Munawar (1986) supplied data for phytoplankton and zooplankton, and unpublished data for forage fish were supplied by J. H. Selgeby (Fish and Wildlife Service, Ashland, WI, pers. comm.); (3) Lake Michigan data from Sprules et al. (1991); (4) the Scotian Shelf, with phytoplankton data from Sheldon et al. (1972), zooplankton data from McLaren et al. (1989), benthic data in Fig. 4B from Schwinghamer (1985), and fish data aggregated from documented abundance estimates by age and size of the major commercial species landed in NAFO statistical area 4X for the calendar year 1980 (O'Boyle and Wallace 1986; Campana and Simon 1986; Stephenson et al. 1986; McGlade et al. 1986).

The Scotian Shelf detailed phytoplankton data of Fig. 4A are for a single November sampling; the Schwinghamer samples for Browns Bank are similarly based on only a single sampling occasion. The other spectral data are based on either a complete population sample, as in Chadwick's study, or on extensive, year-round sampling, sometimes over several years (Appendix).

Deductions from the Detailed Data

The dominant pattern is displayed by the extensive data for the inland lakes and Lake Michigan. There are three distinct domes characterizing the phytoplankton, zooplankton, and fish. The data sets for all four areas suggest that peaks of the phytoplankton and zooplankton are separated by 4 to 5 orders of magnitude in body mass. The fish peak on the Scotian Shelf appears to be shifted to the right of that for the three lake ecosystems, indicative of the larger sizes of fish found in the marine system. The fish peaks are separated from the zooplankton peaks by about 6 orders of magnitude in body size.

The body size spacing between organism groupings is suggestive of relative sizes of predators and prey, although as pointed out by Conover and Huntley (1980), in any one biomass peak there will be an interweaving of different feeding strategies, reflecting a trophic matrix rather than a direct trophic transfer. Detailed consideration of the ratio of the size of zooplankton predators to their phytoplankton prey was undertaken by Conover (1978) who suggested that 5 to 6 orders of magnitude is not uncommon, with a considerable range depending on circumstances. For fish predators, Kerr (1974) and Jones (1978) provided a number of estimates from which it appears

that predator–prey spacing is generally less, more commonly between 3 and 5 orders of magnitude in relative body masses. Extremes, such as are known from antarctic ecosystems, where spacings in body size between populations of krill and whales are of the order 9, appear to depend on the unique concentrations and distributions of the food particles (Conover 1978).

On the basis of existing information, the size gap in Fig. 4A between the marine fish and zooplankton seems too large to represent a continuous trophic flow. In this case the available benthic data in Fig. 4B provide an obvious explanation. Schwinghamer's (1985) data show, between log body mass of −9 log kcal to +1 log kcal, a broad dome in the invertebrate biomass with a peak at body size of 0 log kcal. This peak, at sizes representing the crustacean and polychaete infauna that are important food items for many of the demersal fishes, fills the gap between fish and zooplankton sizes. We conclude that for the Scotian Shelf the addition of the benthic samples appears necessary to complete the interpretation of the spectrum as a trophic energy cascade.

Figure 4B includes a spectrum for the inshore, intertidal region of the upper Bay of Fundy, quite removed from the Browns Bank area, but included because it is based on multiple samples at approximately bimonthly intervals over 1.5 yr. As in small freshwater lakes, larger species are missing from this restricted environment. However, the spectrum encompasses a bacterial maximum at smallest sizes, a second, somewhat larger peak that can be characterized as the meiofaunal group, and at least one group representing the smaller of the large invertebrates. The single sampling for Browns Bank shows a similar biomass profile, but with a greatly pronounced trough. The apparent stability of the benthic patterns, complementarity between the phytoplankton peak of Fig. 4A and the trough in the benthic spectrum of Fig. 4B, and the fact that the larger infaunal invertebrates fill the gap between zooplankton and fish support Schwinghamer's (1985) speculation of coupling of energy flows between pelagic and benthic ecosystems for this region. Thus the succession of domes in the Scotian Shelf data appears to represent significant features of the underlying trophic organization within a relatively flat overall biomass spectrum.

The four biomass spectra in Fig. 4A also show consistency in their relative levels. The four peaks in the Scotian Shelf spectrum are generally higher than those for Lake Michigan and Lake Superior which are in turn higher than peak concentrations in the inland lakes. That is, while biomass density domes provide information on the trophic organization, their amplitudes over all trophic positions establish what appear to be common, low primary slopes of the spectrum with intercepts representative of specific environments. This interpretation can be tested by comparison with data from other sources.

Extension to Other, More General Sample Data

It is possible to develop biomass spectra for areas where detailed data are not available, provided we have information on the ratios of peak to average biomass density for trophic positions and independent information on the size ranges of organisms involved. In Fig. 2, using the total biomass density and the number of body size classes, we calculated environmental means for several observed domes. In all cases, it was close to the peak in the detailed data. Data from Fig. 1, 2, and 4 can be used to determine size ranges for the different trophic positions. For example, in these figures, the domes of biomass density for zooplankton generally span four size classes, and we use this size range in calculating the environmental mean.

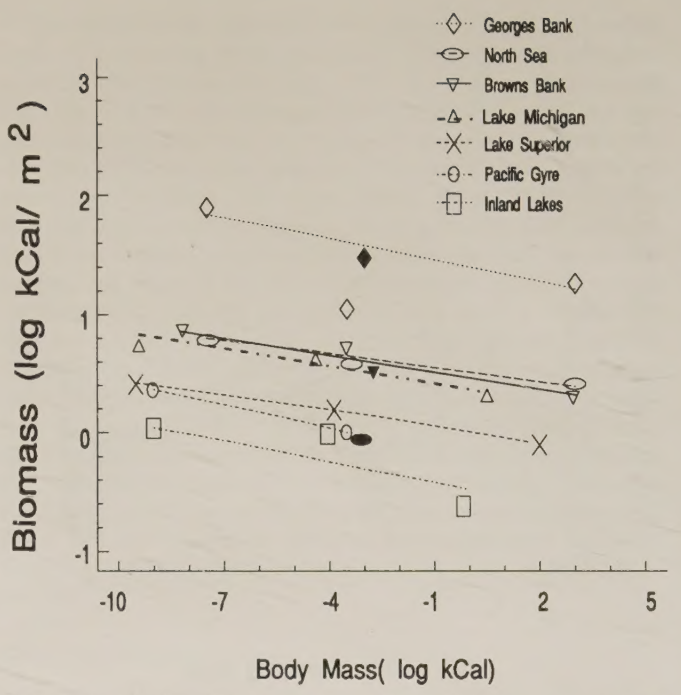

FIG. 5. Standardized biomass spectra for pelagic ecosystems using summary mean points for phytoplankton, zooplankton, benthos, and fish groups. benthos points are represented by solid symbols. The linear regression for each environment is calculated using the sum of zooplankton and benthos as the intermediate point.

The results of applying these rules to data for a total of seven ecosystems are shown in Fig. 5. For the Scotian Shelf, we have replaced the single detailed samples for phytoplankton and the shelf-wide zooplankton sampling data with the available size-aggregated data from a more extensive seasonal sampling (Koslow et al. 1989) of the western shelf around Browns Bank. We have also added spectra for three additional environments: the Pacific Gyre, derived from data in Fig. 1, and two coastal ecosystems of particular interest to fisheries, Georges Bank and the North Sea. Georges Bank data are derived from a recent major compendium of information for this area (Backus and Bourne 1987), together with benthic data from Jones (1984). North Sea phytoplankton and zooplankton data are from Steele and Henderson (1977), and fish data are from Brander and Dickson (1984).

A linear regression through points representing trophic positions within each of the environments in Fig. 5 is used to calculate seven primary spectral slopes and intercepts (Table 1). Benthic data are available for three of them and have been utilized in fitting the lines as indicated below.

Comparative Ecology Using the Biomass Spectra

The most striking feature of Fig. 5 (and Table 1) is the parallelism of the empirical regressions among areas. This parallelism results primarily from the relation between the phytoplankton and fish data. Data at intermediate sizes, consisting of both zooplankton and benthos concentrations, are more variable. Variations in relative zooplankton densities were also observed by Sprules and Munawar (1986) for the Great Lakes.

The highest biomass densities of all seven environments are found on Georges Bank. Benthic data for the bank are close to the fitted line, but zooplankton concentrations are less than 40% of the benthic concentrations, a fact noted and discussed by

TABLE 1. Statistics of biomass spectra for seven environments. The regression coefficients are for the equation $\log B = a + b \log M$ for the biomass spectra in Fig. 5. The last column gives the correlation coefficient r.

Environment	Coefficient		
	a (SE)	b (SE)	r
Georges Bank	1.41 (0.08)	−0.06 (0.02)	−0.97
North Sea	0.50 (0.03)	−0.04 (0.01)	−0.99
Browns Bank	0.50 (0.06)	−0.05 (0.01)	−0.86
Pacific Gyre	0.22 (—)	−0.07 (—)	—
Lake Michigan	0.34 (0.08)	−0.05 (0.01)	−0.94
Lake Superior	−0.01 (0.03)	−0.04 (0.01)	−0.99
Inland lakes	−0.49 (0.26)	−0.06 (0.05)	−0.79
Within ecosystems	0.21 (0.04)	−0.05 (0.01)	−0.88
Overall species (Boudreau and Dickie 1989)	0.66 (0.15)	−0.04 (0.05)	−0.01

Riley (1956) and Jones (1984). By contrast, the North Sea biomass spectrum, which is similar to that of Browns Bank, is closely fitted by all three points when zooplankton are used as the midpoint. Benthic concentrations for the North Sea are only 20% of the zooplankton. The Browns Bank densities, which are generally comparable in level with the North Sea, have zooplankton and benthos present about equally, both being close to the fitted spectral line.

If a linear biomass density spectrum represents a normal aquatic trophic cascade, the dissociation of the midpoint of the spectrum into what appears as its two chief components suggests that the trophic flow supporting the fish biomass on Georges Bank involves the benthos as the main linkage. By contrast, the North Sea system appears to be much more dependent on trophic linkages involving the zooplankton. The Browns Bank area, as mentioned above, appears to involve about equal elements of zooplankton and benthos.

This interpretation is supported by what we know of the physical oceanography of the three areas. The relatively shallow Georges Bank is the centre of a vigorous tidally generated gyral circulation that prevents stratification at any time during the year (Loder and Greenberg 1986). The northern North Sea is a basically stratified system during most of the growing season. Browns Bank is intermediate, the shallow depth inhibits stratification when tidal effects govern the circulation, but the gyre formed is too weak to be maintained during storms, at which time the area is covered by stratified waters from the surrounding shelf (Smith 1989). This interpretation of the intermediate biomass peaks in relation to physical conditions is also appropriate for the three freshwater systems, which appear to be typically stratified systems and where our data suggest that the zooplankton biomass acts as the main trophic energy carrier.

This connection between hydrographic information and routes of the trophic energy flow offers a mechanism explaining differences in average biomass levels among the illustrated systems (Cushing 1989). The gradations between the high biomasses on Georges Bank to the low biomass of the inland lakes seem to be in keeping with general knowledge of the basic supply of nutrients as indicators of fertility among the systems (Nixon 1988). That is, nutrient availability in conjunction with the degree of activity of energy transfers within the ecosystem determines the primary level of the biomass spectrum.

TABLE 2. Statistics for the intercepts, in terms of log biomass density, of partial Great Central Lake spectra before and after treatment with fertilizers.

	Untreated	Treated	% change
Phytoplankton	0.60	0.71	30
Zooplankton	−0.56	0.24	520
Juvenile salmon	−0.95	−0.85	30

The importance of nutrient supply on the level of the spectrum is supported by data from LeBrasseur et al. (1978) who reported the effects of moderate artificial fertilization of Great Central Lake, a relatively large natural lake on Vancouver Island. The data do not include all the fish species in the lake and so cannot be used for Fig. 5; however, data for phytoplankton, zooplankton, and juvenile salmon densities are summarized in Table 2 for treated and untreated periods. The data show twofold increases in the phytoplankton and in the density of young salmon with nutrient application, although, in keeping with the Fig. 5 variability at intermediate trophic levels, the zooplankton increased eightfold. This suggests that alternative intermediate pathways of trophic energy flow may exist or that zooplankton may be particularly sensitive indicators of non-equilibrium states.

We have summarized the data of Fig. 5 in Table 1, calculating regressions from three points for each ecosystem. The intermediate zooplankton and benthic biomasses each span four size groups when they are plotted separately, but together they span five size classes, so that their summed point is only slightly above the higher of the two separate points. We have combined zooplankton and benthos biomass data for an intermediate point when data for both were available. The results show that the slopes for the seven environments are all significantly negative but where comparable statistics are available are not significantly different from each other. This low, common, within-environment slope is not significantly different from the slopes calculated by Borgmann (1987), Minns et al. (1987), and Boudreau and Dickie (1989) using data for individual species from a variety of situations.

Discussion

The variations in the heights of the various spectral intercepts in Fig. 5, in relation to what is known about the environments in which they appear, support the thesis that the average overall biomass density is a function of the availability of nutrients to the system, rather than a function of the structure of the particular biological community that exists in it. In light of the theory, these observations also support the thesis (Platt et al. 1984) that average production per unit area within an ecosystem should be predictable from observations of the average biomass density in any of the trophic positions that are a part of it.

It is more difficult to rationalize the uniform low values for the empirical slopes of these spectra. The original descriptions of the spectrum by Sheldon et al. (1972) related its slope to the turnover times of organisms of different body sizes. Platt and Denman (1978) identified specific metabolic components in the allometric equation underlying the spectral slopes and deduced

a possible general slope value from them. Subsequent investigations (Dickie et al. 1987; Boudreau et al. 1991; M. L. Thiebaux and L. M. Dickie, unpubl. data) have recognized the importance of individual organism metabolism on the slope parameters, but in addition have associated it with the nature of the predator–prey linkages and the apparently necessary population density adjustments that mediate population energy flow in relation to individual food requirements.

The values found here for seven different ecosystems (Table 1) are the same as those calculated by Borgmann (1987) and Minns et al. (1987) and found for species averages from different environments by Boudreau and Dickie (1989). Sprules and Munawar (1986), on the basis of data on only the phytoplankton and zooplankton parts of the spectrum from several areas, found variability in slopes, which they tentatively attributed to the nature of the biological systems. Platt et al. (1984), using Pacific Gyre data confined primarily to the lower trophic positions, also found a higher slope than is shown here.

Some of the uncertainty can be resolved by enquiring about consistency of the observed primary spectral slopes with the requirements for conservation of energy transfer (Denman et al. 1989). Specifically, it is clear that total energy flux in a system is reflected in the respiration per unit area, R/A. If we recognize that this flux is the product of the energy turnover in individual organisms and changes in organism density, then we may write the basic energy equation as

$$(1) \quad R/A = R/N \times N/A$$

where R/N is the respiration per individual at given size and N/A is their corresponding numerical density. Allometric values, which relate R/N to the biomass spectrum, are generally derived from respiration experiments with individual organisms ($\propto w^{0.75}$; Hemmingsen 1960). In this analysis of the spectrum, we take allometric values for the density term, N/A, directly from the empirically fitted biomass spectrum itself. From Fig. 5 and Table 1, this is the slope of the numerical spectrum, that is, the slope of the normalized biomass spectrum (biomass density divided by body size). Whence the average slope, -0.05, from Table 1 indicates that $N/A \propto w^{-1.05}$. Thus from eq. (1):

$$(2) \quad R/A \propto w^{0.75} \times w^{-1.05} \propto w^{-0.30}.$$

That is, total respiration per unit area falls with increasing body sizes of the organisms.

Boudreau and Dickie (1989) found empirically that for the entire community of organisms in an ecosystem, production density $P/A \propto w^{-0.23}$. We thus have the result that production efficiency

$$(3) \quad \frac{P/A}{R/A} \propto w^{-0.23} \times w^{0.30} \propto w^{0.07}.$$

That is, the low slope of the biomass spectrum suggests that with increases in the body sizes of the organisms there is a slight increase in production efficiency, P/R.

Existing data do not provide measures of P/R sensitive enough to statistically test this deduction. The data of Humphreys (1979) show that there are differences in average P/R among major organism groups such as trophic positions; however, the size ranges within any observed position are large and so dependent on the particular species for which data were available that it is difficult to infer a relation between P/R and body size from them.

On the other hand, the steeper slope of the primary spectrum deduced by Platt and Denman (1978) (-0.22), while consistent with the data of Beers et al. (1975, 1982) reanalyzed by Platt et al. (1984), is being compared with a partial spectrum. Since in eq. (1) terms, they used the same value of R/N as we do, the difference between their results and ours derives entirely from different estimates of the density flux term N/A. They used an a priori theoretical measure of numerical density change, N/A, derived from Fenchel's (1974) estimates of the weight-dependent maximum intrinsic rate of (numerical) increase. The difference from our empirical fittings may thus partly reflect discrepancies between the expected potential size-related rate of change in abundance and observed average densities. That is, realized density changes are less than are implied in the potential rate of reproduction. We conclude that the steep slope displayed by Beers' partial spectra for microplankton may rather reflect the variability displayed in intermediate trophic positions. Our slope estimates for the Pacific Gyre, based on a standardized replot of the largely phytoplankton and zooplankton data, are also somewhat higher than slopes for the other areas, in keeping with observations of Sprules and Munawar (1986) on partial spectra.

In balancing the energetics equation, we have thus been left with the reasonable deduction (eq. (3)) that densities of larger aquatic organisms may have a slightly increased production efficiency. With this proviso, the empirically measured slope of the standardized primary spectrum of -0.05 seems to reflect the general dynamics of ecosystem production and efficiency, that is, the observed changes in biomass density and respiration density with body size are consistent with considerations of conservation of energy transfer.

We need to understand the significance of variations displayed at intermediate levels of the trophic cascade, which affect the interpretation of partial spectra. Steele (1974) demonstrated that phytoplankton sampling tends to spatially average over various phases of the predator–prey relations that operate throughout the field of occurrence of a spring bloom, hence integrating over details of the underlying dynamics. Similarly, sampling of long-lived fishes generally cuts across several cohorts, leading to time averages of the fish biomasses. Annual sampling for these groups may therefore meet the criteria for adequate averaging of the dynamics (Denman et al. 1989). By contrast, the time and space scales of biological sampling for the intermediate zooplankton and benthos seem to be closest to those of their population dynamics. This gives rise to a higher likelihood of aliasing of the sampling results with the various driving forces. It could also mean, however, that requisite sampling of intermediate scales in the trophic linkages might enable their use as especially responsive indicators of ecosystem change or ecosystem health (Kerr and Dickie 1984).

The presence of a relatively flat and uniform primary spectral slope among all the environments seems at first to be at variance with the conclusions of early reasoning about models of trophic chains, which suggested that successive levels of predators should show an alternation of abundance, depending on whether controls on abundance are exercised by the basic food production or by predator requirements (Hairston et al. 1960). Catch by a predator depends in the short term on local availability of prey which is a function of its average density and degree of aggregation rather than its abundance (Boudreau 1992). In the long term, it is the rate of replacement of prey implied by production and redistribution of the prey abundance after predation that is important. What we wish to emphasize here is that the uniformity of the spacing and height of domes in the biomass spectrum is designed to reflect their local density, not their abundance. We conclude that these average densities, while apparently reflecting population properties related to food

requirements of predators, are not directly indexing the abundance changes usually implied in population models, and hence that our deductions are not at variance with them.

Dickie et al. (1987) and Boudreau and Dickie (1989) pointed out that the ubiquity of the secondary scaling of production and biomass density by size must reflect a relatively rapid adjustment in the density that enables predators to meet their foraging requirements. Thus, while the uniform slopes of the primary spectrum reflect long-term mechanisms controlling production, the secondary peaks must depend on mechanisms operating on relatively short response scales. It follows that the effects of short-term perturbation on ecosystem functioning and yield need to take into account the detailed structural features at individual trophic positions (M. L. Thiebaux and L. M. Dickie, unpubl. data). That is, it is in the secondary structuring of the spectrum, and particularly in the zooplankton and benthos, that one might expect to find reflections of year-to-year changes in ecosystems.

It follows that effects that may perturb individual food chain elements of ecosystems, such as the abundance of individual species of invertebrates or fish, are likely to be reflected in measures of aggregation or density separately from their effects on overall abundance (Paloheimo and Dickie 1964). The opportunity afforded here of comparing the fish density information with that of other trophic components in the same environments enhances appreciation of the practical implications. For example, difficulties in establishing the levels of organism densities representing intermediate trophic stages in the sampling on Georges Bank, North Sea, and the Scotian Shelf suggest that difficulties in detecting or predicting yield changes may result from deficiencies in the sampling of the population distribution. Similar problems attend the estimation of the average density as opposed to average abundance of fish stocks. Given the conclusion that it is possible to generalize production potential on the basis of the density information contained in the biomass density spectrum, it becomes clear that the outstanding problem of applying this knowledge to yield management becomes that of adequately sampling the area of distribution of the trophic components to estimate total flux or abundance (Crecco and Overholtz 1990).

Acknowledgements

We are grateful for comments and advice on earlier versions of this manuscript by S. R. Kerr, T. Platt, P. Schwinghamer, and S. E. Campana. J. H. Selgeby kindly provided data from his unpublished sampling for fish along the south shore of Lake Superior.

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Appendix. Algorithms for Standardization of Published Data

The original expression of biomass and body size accords with sampling methodologies and aims of particular studies.

Part (i) lists the conversion algorithms used in the standardization. In cases where the original publications do not provide necessary data, reasonable assumptions are made from comparable situations. The list is by ecosystems and gives the original units, the conversion algorithms, and the average depth used in this paper to convert from volumetric to area measures of density.

All measurements are in wet weight unless specified. DW, C, and ESD stand for dry weight, carbon, and equivalent spherical diameter, respectively.

Superscripts refer to the numbered reference lines in the source list in Part (ii).

Codes for conversion algorithms for body size refer to items listed in Part (iii) and for biomass to items listed in Part (iv).

Part (i). Table of Data

Species group	Size: Source data*	Size: Conversion algorithms	Biomass: Source data	Biomass: Conversion algorithms	Depth (m)
		Scotian Shelf			
Phytoplankton	ESD (μm)[a]	A	ppm[a]	I	100
C. finmarchicus	μg DW[b]	B	N m^{3} [b]	V	100
Other copepods	μg DW[b]	B	$\mu g/m^{3}$ [b]	VI	100
		Browns Bank			
Phytoplankton	ESD (μm)[a]	A	mg Chl *a*/m^{2} [c]	II	
Zooplankton	μg DW[b]	B	mL/m^{2} [c]	III	
Benthos	ESD (μm)[d]	A	mm^{3}/m^{2} [d]	III	
Fish	g[e]	C	Tons[e]	IV	
		Inland lakes			
Phytoplankton	ESD (μm)[f]	A	μg/L[f]	VII	5
Zooplankton	ESD (μm)[f]	A	μg/L[f]	VII	7.8
Fish	g[g]	C	kg/ha[g]	IV	
		Lake Superior			
Phytoplankton	μg[h]	C	(μg/L)/μg[h]	VIII	20
Zooplankton	μg[h]	C	(μg/L)/μg[h]	VIII	50
Fish	g[i]	C	g/ha[i]	IV	
		North Pacific Central Gyre			
Phytoplankton	ESD(μm)[j]	A	no./L[j]	IX	200
Zooplankton	μg C[k]	D	(μg C/L)/μg C[k]	VIII	100
		Thames River			
Fish	g[l,m]	C	g/$m^{l,m}$	IV	
		Great Central Lake			
Phytoplankton	IL	—	mg Chl *a*/m^{2} [n]	II	
Zooplankton	IL	—	g/m^{2} [n]	IV	
Fish	mg[n]	—	mg/m^{2} [n]	IV	
		Lake Washington			
Fish	g[o]	C	kg[o]	IV	
		Georges Bank			
Phytoplankton	Browns	—	no./L[p]	V	100
Zooplankton	Browns	—	mg C/m^{3} [f]	IX	100
Benthos	Browns	—	kcal/m^{2}[q]	—	
Fish	Browns	—	Tons/km^{2}	X	
			Landings[p]		
		North Sea			
Phytoplankton	Browns	—	mg Chl *a*/m^{3} [r]	II	50
Zooplankton	Browns	—	g DW/m^{2} [r]	VI	
Benthos	Browns	—	g/m^{2} [q]	VI	
Fish	Browns	—	kg/ha	X	
			Landings[s]		

*IL indicates the use of inland lakes size data. Browns indicates the use of Browns Bank size data.

Part (ii). Source List

a, Sheldon et al. 1972.
b, McLaren et al. 1989.
c, Koslow et al. 1989.
d, Schwinghamer 1985.
e, O'Boyle and Wallace 1986; McGlade et al. 1986; Campana and Simon 1986; Stephenson et al. 1986.
f, Sprules et al. 1983.
g, Chadwick 1976.
h, Sprules and Munawar 1986.
i, J. H. Selgeby, Fish and Wildlife Service, Ashland, WI, pers. comm.
j, Beers et al. 1975.
k, Rodriguez and Mullin 1986.
l, Mathews 1971.
m, Mann 1965.
n, LeBrasseur et al. 1978.
o, Eggers et al. 1978.
p, Backus and Bourne 1987.
q, Jones 1984.
r, Steele and Henderson 1977.
s, Brander and Dickson 1984.

Part (iii). Conversion Algorithms of Body Size to kcal

A. From ESD:
1. Convert to ESD in m.
2. Equivalent spherical radius = $ESD \cdot 2^{-1}$.
3. Volume = $4/3 \cdot \pi r^3$.
4. Assume 1 cc = 1 mL = 1 g = 1 kcal (Banse and Mosher 1980).

B. From DW:
1. 1 g DW = 6 kcal (McLaren et al. 1989).

C. From wet weight (Banse and Mosher 1980):
1. For fish, assume 1 g = 1.3 kcal.
2. For all other species, assume 1 g = 1 kcal.

D. From C (Backus and Bourne 1987):
1. 1 g C = 10 kcal.

Part (iv). Conversion Algorithms of Biomass Density to kcal/m^2

I. From ppm:
1. 1 ppm = 1 cc/1 m^3
2. Multiply by sampling depth.

II. From Chl *a*/m^2:
1. Assume a carbon to chlorophyll ratio of 54 (Widdows et al. 1979).
2. Convert to kcal using D.

III. From mm^3/m^2:
1. Convert to cc.
2. Convert to kcal using A.

IV. From kg/ha:
1. Convert to g and m^2.
2. Convert to kcal using C.

V. From no./m^3:
1. Multiply by size in kcal.
2. Multiply by sampling depth.

VI. From μg dry weight/m^3:
1. Convert to kcal using B.
2. Multiply by sampling depth in m.

VII. From μg/L:
1. Convert to g and m^3.
2. Multiply by sampling depth.

VIII. From (μg/L)·(Δ μg):
1. Multiply by Δ μg.
2. Convert to g and m^3.
3. Multiply by sampling depth.
4. Convert to kcal using C.

IX. From μg C/volume:
1. Convert to kcal using D.
2. Convert to m^3.
3. Multiply by sampling depth.

X. From landings in tons/km^2:
1. Convert to g and m^2.
2. Divide by landing/biomass (*L*/*B*) ratio of 0.26 (Boudreau and Dickie 1989).
3. Convert to kcal using C.

Effect of Population Density on Gillnet Catchability in Four Allopatric Populations of Brown Trout (*Salmo trutta*)

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Borgstrøm, R. 1992. Effect of population density on gillnet catchability in four allopatric populations of brown trout (*Salmo trutta*). Can. J. Fish. Aquat. Sci. 49: 1539–1545.

The gillnet catchability of allopatric brown trout (*Salmo trutta*) populations was studied in four Norwegian high-mountain lakes, with large differences in population densities. The number of fish and the length composition of the populations were estimated by mark–recapture. The catchability coefficient and the catch per unit effort were described by power functions, and the catchability was inversely related to number of fish present. An important fisheries management implication is that brown trout populations with low densities are more vulnerable to gillnets than populations with high densities. Furthermore, the use of gillnet catches as an estimator of brown trout population density will be biased.

On a étudié la capturabilité aux filets maillants de populations allopatriques de truite brune (*Salmo trutta*) peuplant quatre lacs de haute montagne de la Norvège et montrant d'importantes variations de densités. On a déterminé le nombre de truites et la répartition des longueurs dans chaque population à partir de données de marquage et de recapture. Des fonctions de puissance ont servi à la description du coefficient de capturabilité et des prises par unité d'effort; ainsi, la capturabilité était en relation inverse au nombre de poissons présents. Le fait que les populations de truite brune de faible densité soient plus vulnérables à la capture dans des filets maillants que des populations à densité élevée constitue une importante conséquence en ce qui concerne la gestion des pêches. De plus, l'utilisation de données sur les prises aux filets maillants comme estimateur de la densité d'une population de truite brune sera biaisée.

Received April 18, 1991
Accepted February 10, 1992
(JB007)

Reçu le 18 avril 1991
Accepté le 10 février 1992

Gill nets are commonly used in commercial fisheries, and in Scandinavia in recreational fisheries as well. Moreover, gill nets are an important tool in assessment of fish population parameters (e.g. Hamley 1975). In some studies on catchability by gill nets, an inverse relationship between stock size and the catchability coefficient has been observed (e.g. Henderson et al. 1983; Crecco and Savoy 1985; Angelsen and Olsen 1987). A similar relationship has also been found for sport fisheries (e.g. Bannerot and Austin 1983). The increase in catchability with decreasing stock size has been explained by a nonrandom search behavior of commercial fishermen coupled with the ability of the stock to contract its geographical distribution when stock declines (Paloheimo and Dickie 1964; Crecco and Overholtz 1990). However, there may be additional explanations, at least for freshwater species. The probability of catching fish by gillnetting is partly a function of the probability of encounter (Hamley 1975). This probability is likely to be proportional to the distance travelled by the fish during the sampling period (Rudstam et al. 1984). Because swimming speed and search pattern of fish may depend on the available prey density distribution and prey types (Persson 1987, 1990), this implies that a change in catchability may be related to a change in prey availability and/or in feeding behavior. Therefore, the gillnet catchability may be a function of population density through its effect on food availability and search behavior of the fish. This explanation has hitherto not been pursued. The objective of the present study was to investigate the relationship between number of fish and the gillnet catchability in four allopatric brown trout (*Salmo trutta*) populations with different densities.

Methods

Lakes

It would be impossible to obtain a large variation in fish population densities and size structures in a single population within a reasonable period of time, and four lakes with natural allopatric brown trout populations were chosen for the study. Lake Kollsvatn, Lake Krokavatn, and Lake Skavatn are situated on the Hardangervidda mountain plateau 1182, 1233, and 1258 m above sea level, respectively. Lake Løyning is located at the western margin of the same mountain area 595 m above sea level. Substituting spatial variation for temporal variation, i.e. the use of catch data from four lakes with different abundance of trout, instead of fishing one population in which the numbers varied substantially by time may be a problem in catchability analysis because of differences in lake morphology and physical conditions. However, all lakes are small, with surface areas in the range 0.45–0.63 km^2. The average depths are in the range 5.7–14.7 m and maximum depths in the range 13–46 m (Table 1). Furthermore, differences in temperature regimes during the sampling periods were small, with mean surface temperatures during the gillnettings in the range 7–11°C (Table 2). As indicated by the high Secchi disc transparencies (Table 1), the lakes are oligotrophic, with low potentials for secondary production.

Population Estimation and Gillnetting

In Lake Kollsvatn, Lake Krokavatn, and Lake Skavatn, abundance of trout were estimated by mark–recapture

TABLE 1. Some characteristics of Lake Løyningsvatn, Lake Kollsvatn, Lake Krokavatn, and Lake Skavatn.

Lake	Height a.s.l. (m)	Area (km^2)	Max. depth (m)	Mean depth (m)	Secchi disc transparency (m)
L. Løyningsvatn	595	0.49	46	14.7	10.5–14.9
L. Kollsvatn	1182	0.63	13	6.5	>13
L. Krokavatn	1233	0.45	16	8.6	16
L. Skavatn	1258	0.48	15	5.7	14

TABLE 2. Experimental gillnetting periods, total effort in gillnet nights, and surface temperatures during sampling in Lake Løyning, Lake Kollsvatn, Lake Krokavatn, and Lake Skavatn.

Lake	Date	Total effort (net nights)	Surface temp. (°C)
Lake Løyning	June 18–21, 1986	34	7.9
	June 19–25, 1987	90	7.6
	June 20–25, 1988	100	10.8
	June 22–29, 1989	120	6.7
Lake Kollsvatn	July 20–26, 1988	108	9.5
	July 28 – Aug. 3, 1989	154	11.0
Lake Krokavatn	Aug. 1–6, 1987	95	10.5
	July 14–20, 1988	124	10.5
Lake Skavatn	July 30 – Aug. 6, 1990	110	8.4

(Chapman's adjusted Petersen estimate) (Ricker 1975). Fish captured with a 50-m-long beach seine (5-mm bar measure) in July, shortly after ice break-up, were marked by removing the adipose fin or by fin-marking with Alcian Blue using a jet inoculator (Hart and Pitcher 1969; Pitcher and Kennedy 1977) and released at the capture site. Capture sites were placed at all shore sections with suitable bottom conditions for beach seining, and in practice spread around the entire shoreline. In Lake Skavatn, fish caught by electrofishing along the shoreline on a stretch with large blocks and boulders were marked as well. To avoid a high number of fish recaptured during the seining, and thus reduce the handling stress of marked fish, the seining and marking were stopped after 5–6 d, when all available seining sites had been used once or twice.

The recapture of marked fish started directly after the seining and marking period and was carried out with 25-m-long monofilament gill nets, with mesh sizes in the range 16.0–52 mm (bar measure). In the littoral zone, gill nets with height 1.5 m were set on the bottom, while gill nets with height 6 m were set in the pelagic depth ranges 0–6 and 6–12 m, at least 50 m from the shore, and towards the middle of the lakes. Littoral gill nets of each mesh size were set at random, with netting places covering the whole shoreline. Likewise, the pelagic gill nets were set randomly in series consisting of three gill nets with different mesh sizes, with an opening between each gill net.

The number of fish in the following length-classes was estimated: 14.0–19.9, 20.0–29.9, and 30.0–39.9 cm. Population estimates from Lake Løyning are adapted from Borgstrøm (1992).

For the catchability study, only mesh sizes 16.0, 19.5, 22.5, 26.0, 35, and 39 mm were used, except in Lake Løyning, where the two largest mesh sizes were omitted because fish above 26 cm in length were missing. In June 1986, two gangs of gill nets with height 6 m and with mesh sizes 16.0, 19.5, 22.5, and 26 mm set in the pelagic depth range 12–18 m did not capture fish in Lake Løyning. Mesh sizes 22.5 and 26.0 mm in two gangs of gill nets, set on the bottom at depths approximately in the range 10–20 m, captured less than 10% compared with similar gill nets set in the littoral zone, while mesh sizes 16.0 and 19.5 mm were empty. Therefore, these depths were not included in the experimental gillnetting in this lake. In the other lakes, the pelagic nets set in the depth range 6–12 m usually reached the bottom due to the small areas with depths greater than 12 m. To exclude bias in the catchability estimates due to changes in population size caused by mortality, recruitment, and growth in length, only gillnet catches obtained in the first week after the marking period are used in the present study (Table 2).

For each mesh size used in the catchability studies, the catches from littoral and pelagial set gill nets were combined. For length-class 14.0–19.9 cm, one unit of gillnet effort was defined as the combined use of mesh sizes 16.0 and 19.5 mm. For length-classes 20.0–29.9 and 30.0–39.9 cm, the corresponding units were 19.5, 22.5, and 26.0 mm and 26.0, 35.0, and 39.0 mm, respectively. The catch for the entire length range 14.0–39.9 cm was obtained by summing the catches for each length-class.

The catchability coefficient is defined by

$$q = \frac{C_t}{f_t \cdot N_t}$$

where C_t/f_t is the catch per unit of effort during the time interval t and N_t is the mean population present per 0.1 km^2 of lake surface area during the interval (Ricker 1975). N_t is estimated as the arithmetic mean number present between first and last fishing day. It is assumed that the population declines solely due to fishing mortality during this short time interval. Fish abundance is expressed as density per unit area, and not per unit volume of the lakes, mainly because it is unlikely that brown trout as predominantly bottom feeders in the littoral zone are randomly distributed in the whole water body.

The relationships between q and N_t and between C/f and N_t

TABLE 3. Estimated number of brown trout in Lake Løyning, Lake Krokavatn, Lake Kollsvatn and Lake Skavatn, based on number marked, number caught, and recaptures of marked fish. Data from Lake Løyning are adapted from Borgstrøm (1992). S, length-class 14.0–19.9 cm; M, length-class 20.0–29.9 cm; L, length-class 30.0–39.9 cm; T, period of catchability study.

Lake	Year	Length-class	No. seined and marked	No. gillnetted	No. recaptured	Estimated no.	Confidence limits (0.95)	No. caught during T	Mean no. in lake during T
Løyning	1986	S	240	446	30	3475	2460–5081	72	3439
		M	342	417	27	5121	3567–7626	100	5070
	1987	S	216	381	35	2303	1668–3276	237	2185
		M	263	393	39	2600	1916–3624	170	2515
	1988	S	264	361	28	3308	2317–4894	185	3216
		M	92	222	7	2592	1347–5458	91	2547
	1989	S	606	570	57	5976	4693–7878	341	5806
		M	172	263	18	2404	1553–3904	153	2328
Krokavatn	1987	M	12	31	9	42	23–83	13	36
		L	69	118	16	490	309–817	71	455
	1988	M	15	15	5	43	20–98	15	36
		L	29	61	9	186	103–372	31	171
Kollsvatn	1988	S	65	95	13	453	272–802	85	411
		M	310	341	35	2955	2140–4204	318	2796
		L	63	94	5	1013	479–2338	92	967
	1989	S	47	102	8	549	294–1124	94	502
		M	64	172	3	2811	1147–7028	162	2730
		L	86	109	4	1914	854–4785	100	1864
Skavatn	1990	S	103	101	23	442	300–680	83	401
		M	40	65	14	180	110–311	59	151
		L	13	68	3	242	99–604	54	215

TABLE 4. Parameter estimates for the power function $C/f = aN^{1+B}$ for different length-classes of brown trout, with combined data sets from Lake Løyning, Lake Kollsvatn, Lake Krokavatn, and Lake Skavatn.

Length-class (cm)	Parameter	Estimate	SE	t-statistic ($B = 0$)	p
14.0–19.9	a	0.641	0.200		
	B	−0.471	0.047	9.93	<0.001
20.0–29.9	a	0.020	0.023		
	B	−0.017	0.171	0.99	<0.200
30.0–39.9	a	0.442	0.422		
	B	−0.516	0.186	2.77	<0.05
14.0–39.9	a	0.322	0.179		
	B	−0.362	0.078	4.64	<0.001

were examined by fitting the data to the power functions $q = aN_t^B$ and $C/f = aN_t^{1+B}$ (Crecco and Savoy 1985). The PROC NLIN procedure in SAS (1987) was used to find the least-squares estimates for the nonlinear models, with modified Gauss–Newton as the iterative method.

Results

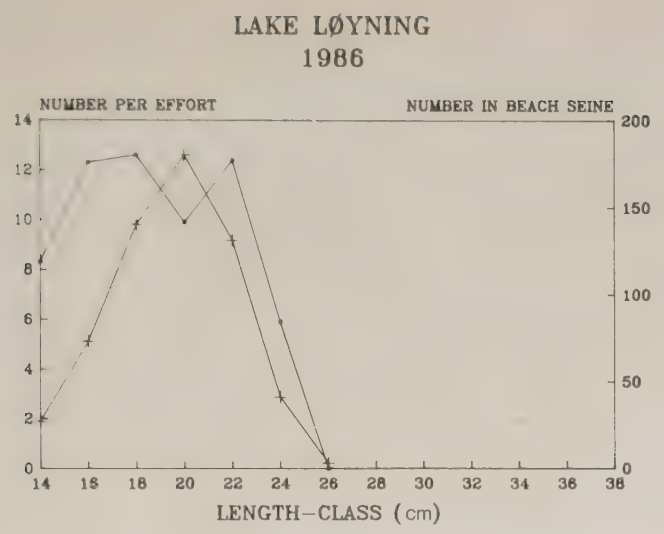
Length Frequency in Beach-Seine and Gillnet Catches

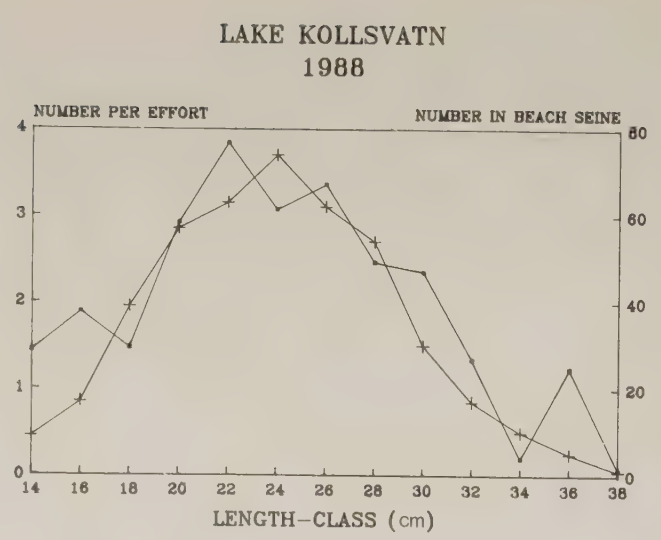
The estimated number of fish in length-classes 14.0–19.9, 20.0–29.9, and 30.0–39.9 cm varied considerably between the four lakes and within the same lake between years (Table 3). These differences between lakes and years are reflected in the length frequency distributions in beach-seine catches and in the catch per unit effort (C/f) obtained by the experimental gill nets (Fig. 1); the length-class which dominates in the lake dominates the beach-seine and the gillnet catches as well. Therefore, differences in C/f observed among lakes and years cannot be explained by the differences in length structures of the populations combined with the retention probability of the gill nets used.

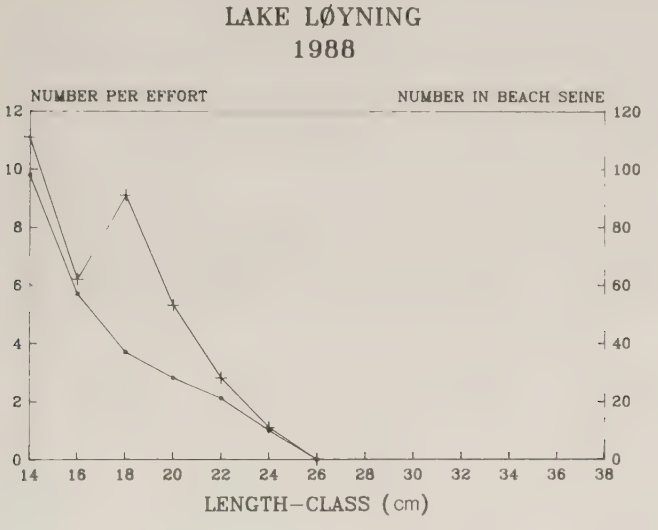
Catch per Unit Effort and Catchability

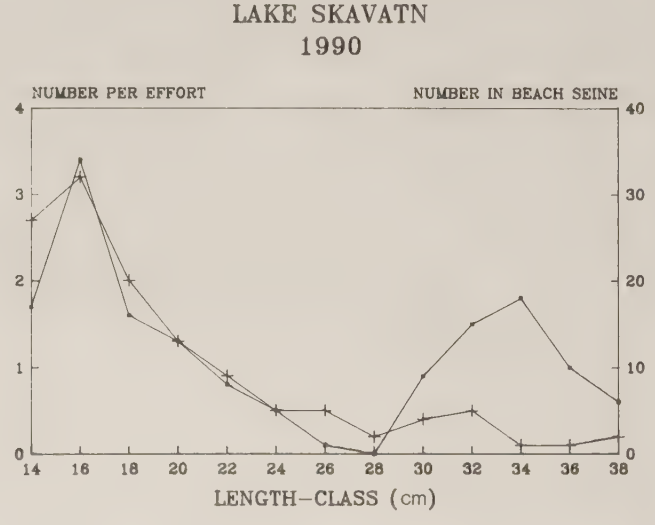
For each length-class, an increase in C/f was found with increasing population density (Fig. 2). The power function $C/f = aN_t^{1+B}$ for the relationship between catch per unit effort and population number (from Table 3) gave a significant fit for length-classes 14.0–19.9 ($p < 0.001$), 20.0–29.9 ($p < 0.001$), and 30.0–39.9 cm ($p < 0.01$) and the whole length range 14.0–39.9 cm ($p < 0.001$) (Fig. 2). The exponent B was significantly less than 0 for length-classes 14.0–19.9 and 30.0–39.9 cm and the entire length range 14.0–39.9 cm (Table 4), i.e. a hypothesis of a linear relationship between C/f and N was rejected. For length-class 20–29.9 cm, a majority of the observations is obtained within a very restricted range of densities, and $B = 0$ cannot be rejected (Table 4).

The power function $q = aN_t^B$ gave a significant fit between the catchability coefficient (q) and N_t for length-classes 14.0–









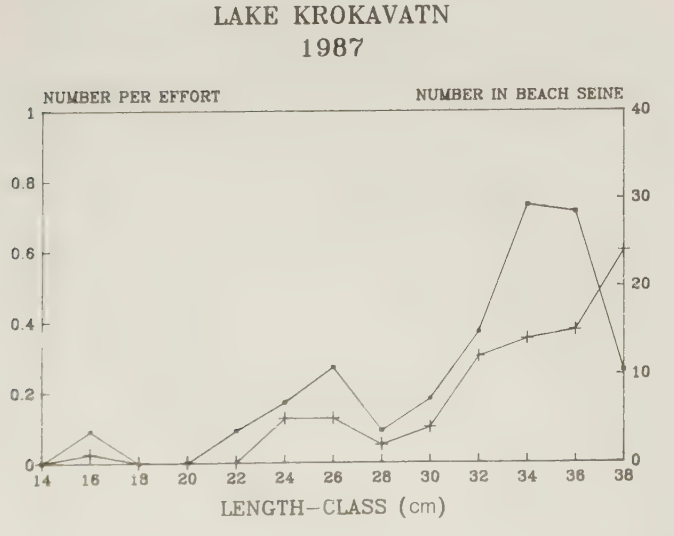


FIG. 1. Length frequency distributions of brown trout sampled by beach seine (×) and experimental gill nets (●) in Lake Løyning (1986 and 1988), Lake Krokavatn (1987), Lake Kollsvatn (1988), and Lake Skavatn (1990).

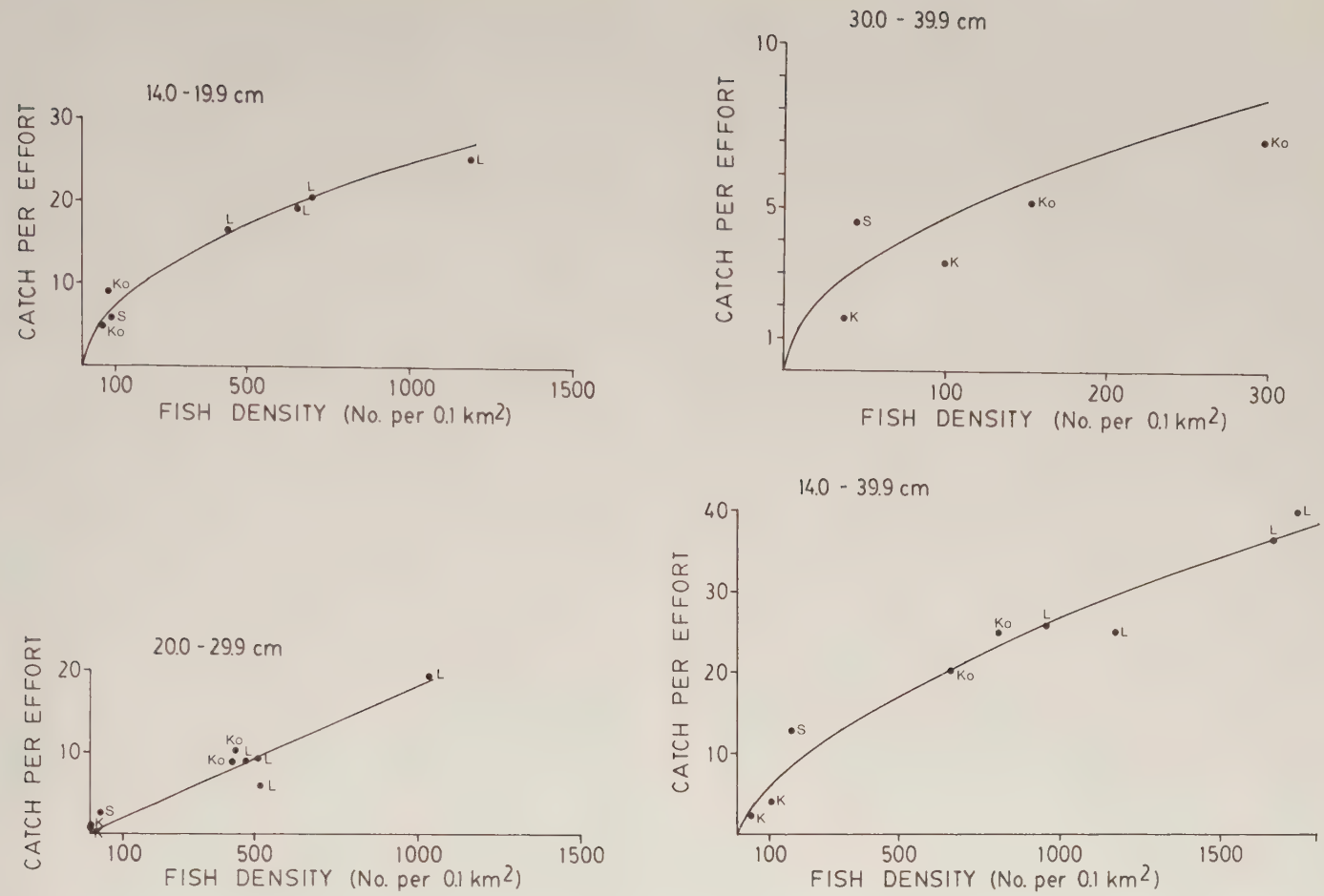


FIG. 2. Relationship between catch of brown trout per unit effort (C/f) and mean population number for three length-classes and the entire length range 14.0–39.9 cm. L, Lake Løyning; Ko, Lake Kollsvatn; K, Lake Krokavatn; S, Lake Skavatn.

19.9 and 20.0–29.9 and the entire length range 14.0–39.9 cm ($p < 0.001$). The fit was not significant for the five observations in length-class 30.0–39.9 cm ($p < 0.1$). Correspondingly, B was significantly different from 0 in the two smallest length-classes and for the entire length range 14.0–39.9 cm (Table 5), and q = constant can be rejected.

Condition Factor

Fulton's condition factor (k) for all length-classes in Lake Løyning was below 0.95 and declined with increasing length (Fig. 3). In Lake Skavatn, k was low for the smallest fish but increased to a maximum above 1.1 for length-classes between 27 and 32 cm. In Lake Krokavatn, the range in k was 1.00–1.15 for trout below 30–33 cm in length, with declining values for larger fish. In 1988, the k was in the range 1.00–1.06 for trout below 25 cm in Lake Kollsvatn, while for larger fish, k ranged from 0.81 to 0.99. In 1989, all length-classes in this lake had lower k values compared with 1988 (Fig. 3).

Discussion

The present study demonstrates that catch per unit effort and catchability by gillnetting were significantly curvilinear, indicating that the catchability coefficient is density dependent. My sampling design with random sampling, combining pelagic and littoral gill nets, virtually excludes the possibility that differences in catchability are the result of differences in habitat use, i.e. a contraction of fish within parts of its distribution area at low population numbers. This is the common reason invoked to explain differences in catchabilities, for example for several marine species (e.g. Pope and Garrod 1975; Maccall 1976; Crecco and Overholtz 1990).

In a study on brown trout, Sømme (1934) captured more than 90% of the catchable fish in 0.3-km^2 Lake Langesjøtjønn in the eastern part of the Hardangervidda mountain plateau by three nights of gillnetting with a daily effort of 30 gill nets. The estimated number of catchable fish present in the lake was 91 and 66 in 1925 and 1933, respectively. Sømme (1941) explained the high catchabilities by an intensive swimming activity of the trout whereby the entire catchable population had passed the same area of the lake during a period of 8–10 d. On the other hand, Jensen (1977) found fish in the dense brown trout population in Lake Øvre Heimdalsvatn to be rather stationary, and he had to move the gill nets every night to maintain high catches. These observations fit well in with the present results, indicating that catchability of brown trout depends on fish behavior and declines with increasing density. Henderson et al. (1983) also found the catchability coefficient in gill nets to be inversely related to population numbers of lake whitefish (*Coregonus clupeaformis*).

The density dependence may be explained in several ways. The encounter probability towards passive gears is a function of fish activity during the sampling period (Rudstam et al. 1984). Therefore, the estimated decrease in catchability by

TABLE 5. Parameter estimates for the power function $q = aN^B$ for different length-classes of brown trout, with combined data sets from Lake Løyning, Lake Kollsvatn, Lake Krokavatn, and Lake Skavatn.

Length-class (cm)	Parameter	Estimate	SE	t-statistic ($B = 0$)	p
14.0–19.9	a	0.618	0.406		
	B	−0.462	0.139	3.33	<0.01
20.0–29.9	a	0.175	0.030		
	B	−0.332	0.054	6.18	<0.001
30.0–39.9	a	0.468	0.807		
	B	−0.524	0.421	1.24	<0.140
14.0–39.9	a	0.118	0.064		
	B	−0.200	0.097	2.07	<0.05

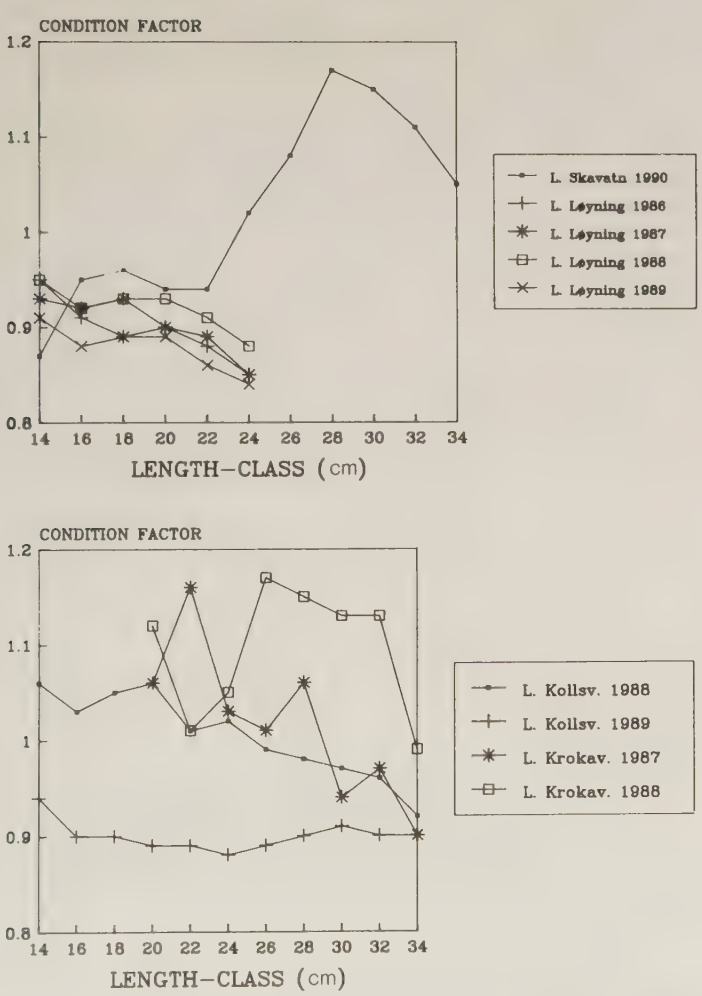


FIG. 3. Mean values of Fulton's condition factor for different length-classes of brown trout from Lake Løyning, Lake Krokavatn, Lake Kollsvatn, and Lake Skavatn.

increasing fish density may be caused by a decline in swimming distances with increasing density of fish. The large differences in observed condition factors, both between the various populations and between size-classes within the same population, may indicate considerable variation in amount of food consumed by the fish in relation to its metabolic requirement (Elliott 1976). Prey species of zooplankton (Hamrin and Persson 1986) and benthic invertebrates (Kajak et al. 1976; Gilinsky 1984) are depressed by fish predation. When food availability increases from an initially low level, swimming speed of fish may increase in response to a higher energy return rate (Ware 1975; Sih 1984; Persson 1990). According to this explanation, it is likely that swimming activity for a specific length-class depends on the total density of the population, through its effects on prey availability. From this follows that estimates of the relationships between C/f and N and between q and N may be most realistic when the fish population as a whole is included in the calculations, i.e. by analysing C/f and q for the entire length range of fish.

The efficiency of a gill net decreases as fish accumulate in it (Hamley 1975). However, the overall low catches per gill net per night obtained in my study, even in lakes with the highest population densities (Lake Løyning and Lake Kollsvatn), suggest a minimal influence of gear saturation upon the catch efficiency.

On an areal basis, Lake Løyning had the most dense population, and also the lowest catchabilities estimated. Because sampling was done in the upper 12 m of all the lakes, the low catchabilities in Lake Løyning could in theory be caused by a dispersion of fish outside the effective range of the gill nets. However, the low numbers of fish captured in deeper parts of the lake do not lend any support to this alternative explanation.

Although the catchability data are obtained by fishing on different populations, it may be concluded that the catchability coefficient for brown trout by gillnetting is density dependent. This density dependence may have important implications both for stock assessments by gillnetting and for fisheries management. Catch per unit effort (C/f) with gangs of gill nets is commonly used as an estimator of fish abundance (e.g. Henderson et al. 1973; Jensen 1977; Ryder 1978). If a linear model between C/f and N is chosen, when catchability varies with fish density, populations with low densities will be overestimated relative to dense populations. Likewise, increased catchabilities with decreasing population density implicate that populations with low densities may be easily overfished, unless catch or gear limits are introduced. For instance, in Norway, such management strategies are not implemented in freshwater fisheries.

Acknowledgements

I thank J. G. Dokk, S. Ingebrigtsen, O. Kambestad, K. Pedersen, and L. Scobie for valuable assistance in the field. Financial support was given by the Norwegian Research Council for Science and the Humanities through the research programme "Dynamics and enhancement of freshwater fish populations" (FFT) and the Directorate for Nature Management. I am also indebted to the Ullensvang Mountain Board for allowing use of gill nets in the lakes, to Jan Heggenes and Lars Rudstam for their constructive criticism of the draft, and to Linda Hjeljord for improving the English.

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Gillnet Selectivity and a Model for Capture Probabilities for a Stunted Brown Trout (*Salmo trutta*) Population

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The gillnet selectivities for a lacustrine population of allopatric brown trout (*Salmo trutta*) were estimated directly, using maximum girth as the parameter for fish size. A model for capture probabilities separating the processes of encountering the net and being retained in case of an encounter was developed. Swimming distances and retention probabilities were estimated from the model. Size selectivities decreased with increasing mesh size and fish size. This was explained by decreasing swimming distances with increasing fish size. The retention probabilities are consistent with a geometrical similarity hypothesis. Serious errors in the assessment of population size structure by gillnetting may arise when variations in swimming distances are not accounted for.

On a déterminé la sélectivité de filets maillants dans le cas d'une population lacustre allopatrique de truite brune (*Salmo trutta*) en utilisant la circonférence maximum comme paramètre de la taille du poisson. À partir de ces données, on a élaboré un modèle des probabilités de capture distinguant la rencontre du filet et la capture dans le filet. Ce modèle a permis de déterminer la distance entre le poisson et le filet ainsi que la probabilité de capture. La sélectivité selon la taille a diminué en fonction d'une augmentation du maillage et de la taille du poisson. Ceci peut s'expliquer par une diminution de la distance entre le poisson et le filet en fonction d'une augmentation de la taille du poisson. Les probabilités de capture concordent à une hypothèse de ressemblance géométrique. D'importantes erreurs dans l'évaluation de la structure des tailles d'une population à partir de données recueillies dans le cadre d'un échantillonnage aux filets maillants peuvent survenir si l'on ne tient pas compte des variations de la distance entre le poisson et le filet.

Received April 18, 1991
Accepted February 10, 1992
(JB006)

Reçu le 18 avril 1991
Accepté le 10 février 1992

Existing models of gillnet selectivities generally do not account for the fact that gillnetting is the combined result of at least two different processes: the fish may encounter the net, and in the case of encounter, it may be caught and retained (Rudstam et al. 1984). Instead, selectivities are described in terms of quantities that are not directly related to these two processes, as for example width and skewness of the selectivity curves (Wulff 1986; Boy and Crivelli 1988; Helser et al. 1990). A review of a number of earlier analyses of gillnet selectivities is given in Hamley (1975).

Several models are based on the assumption that the maximum selectivity for a given mesh size is the same for all mesh sizes. However, direct estimates of gillnet selectivities indicate that large mesh sizes are more efficient than small (Hamley and Regier 1973; Hamley 1980; Borgstrøm 1989; Winters and Wheeler 1990). Rudstam et al. (1984) demonstrated that this could be explained by variations in swimming distances. Therefore, predictions will be biased if equal encounter probability for all size-classes is assumed in situations with strong variation of swimming distance with fish size.

Our objective for the present study was to obtain estimates for the selectivities and develop a model describing the selectivities or capture probabilities based on the separate processes of encounter and retention. Field data were obtained by fishing on a population of brown trout (*Salmo trutta*) with known population structure (Borgstrøm 1992a).

Methods

Field Experiments

The field data were collected in the 0.49-km^2 Lake Løyning, 595 m above sea level, in western Norway (Borgstrøm 1992a). Maximum depth of the lake is approximately 45 m, with mean depth 14.7 m. The lake is oligotrophic, and during June 1985–89 the Secchi disc transparency ranged between 10.5 and 14.9 m, while surface temperature range was 6.7–13.2°C. At 5 m depth the temperature range was 6.4–9.0°C. The only fish species present is a natural population of allopatric brown trout. Due to stagnation of growth in length after maturation, few fish in the population attain lengths above 25 cm, and Fulton's condition factor decreases with increasing length (Borgstrøm 1992b).

To estimate the gillnet size selectivities, we fished with littoral and pelagic gill nets in June 1985–89, using mesh sizes 16.0, 19.5, 21.0, 22.5, 24.0, and 26.0 mm (knot to knot measure). The annual total number of gillnet nights ranged between 41 and 144. In the littoral, the nets were 1.5 m high and 25 m long, while nets used in the pelagic zone at least 50 m from the shore were 6 m high and 25 m long. Mesh size 22.5 mm was not used in June 1985.

All nets were made of monofilament, light grey nylon, with a 0.5 hanging ratio. Single nets were set at each littoral netting locality. In the pelagic zone, a gang of three different-meshed

nets, with an opening between each net, were set at either 0–6 or 6–12 m depth. Each year, the gillnet sampling period lasted for four to six nights in the latter half of June. The sampling was carried out randomly both in the littoral and the pelagic zone down to a depth of 12 m. This limit was chosen because test samplings below 12 m yielded insignificant catches in proportion to catches from the upper 12 m of the lake. The gill nets were set in the evening and lifted the next morning, after 12 h of fishing. The volume of that part of the lake where the pelagic nets were set was 3.5×10^6 m^3. For the littoral nets the volume was 4.5×10^5 m^3.

List of Symbols

G = maximum girth (mm)
g = effective girth, related to G by $g = G - G_0$, G_0 = constant
g_0 = chosen reference girth, $g_0 = 82$ mm
L = total length of fish (mm)
W = weight of fish (g)
m = mesh (knot to knot measure, mm)
M = $4m$ = mesh perimeter (mm)
i = $1, 2, \ldots, I$ = mesh size enumeration subscript
j = $1, 2, \ldots, J$ = size class enumeration subscript
k = $1, 2, \ldots, K$ = year enumeration subscript (omitted if not necessary)
l, p = superscripts denoting littoral and pelagic, respectively
C_{ijk} = catch of size-class j in nets of type i in year k
N_{jk} = number of fish in size-class j in year k
S_{ij} = selectivity of mesh i relative to size-class j
E_i = $n_i A_i$ = effort in nets of type i = number of nets times area of net i
P_{ij} = probability of capture (encounter and retention) of fish j in any net i during one sampling period (i.e. one night)
p_{ij} = probability of encounter per unit effort and per swimming distance covered
Q_{ij} = probability of retention (in case of encounter) of fish j in net of type i
V_j^c = cruising volume of a single fish in size-class j during one night
V_i^s = volume of that part of the lake where nets of type i are set
V_{ij}^{cs} = the intersection of V_j^c and V_i^s
D_{jk} = average swimming distance of a fish j in V_j^c during the sampling period
q = maximum retention probability for the reference girth g_0
s = scaling exponent.

Population Parameters

The weight and total length of all captured fish were measured to the nearest gram and millimetre, respectively. The catchability of a specific length-class is influenced by changes in the condition factor (Winters and Wheeler 1990), and instead of using length as a size parameter in our analysis, we described the fish by its maximum girth. In June 1989, the circumferences of 566 fish were measured just in front of the dorsal fin by a tape measure and defined as the maximum girth. The relationship between maximum girth G, L, and W could be described by the equation

$$(1) \quad G = c\, L^a W^b$$

with $c = 100.4$, $a = -0.386$, and $b = 0.456$ ($r^2 = 0.96$). The length–weight data for fish captured in 1985–88 were then converted to maximum girth according to Eq. (1).

The numbers of fish in each length-class in the population (from Borgstrøm 1992a) were arranged in 5-mm girth-classes from 50–54 to 115–119 mm, in proportion to the frequency of each girth-class within the length-class. The total number of fish in each girth-class was then obtained by summation over all length-classes.

Development of the Model

The size selectivity index is defined according to Hamley (1975) as

$$(2) \quad S_{ij} = \frac{C_{ij}}{E_i N_j}.$$

To develop a model for the capture probabilities, we consider a fish in size class j swimming a distance D_j along a straight-line path in a lake where only one net (of type i) is set. In this simplified situation the fish will encounter the net at most once, and the probability of the fish getting caught in the net is the product of the encounter probability p_{ij}, E_i, D_j, and Q_{ij}:

$$(3) \quad P_{ij} = p_{ij} E_i D_j Q_{ij}.$$

However, when several different nets are set, or the path is curved, we cannot expect this simple formula to hold. The fish may have encountered several nets or the same net many times before getting caught. The path of the fish during the sampling period can then be approximated by a sequence of R straight-line legs of equal lengths. If R is sufficiently large and the nets are set in straight lines at random within V_i^s, the capture probability for each leg is given by Eq. (3). We shall consider the limit $R \to \infty$.

We assume that p_{ij} depends on the swimming pattern of the fish as well as where and how the nets are set, but not on the properties of the nets. For Q_{ij} the situation is just the opposite: under identical external conditions, Q_{ij} presumably only depends on the individual properties of fish and mesh, not on the position along the path; then, p_{ij} and Q_{ij} are constant along the path. Before generalizing Eq. (3), we derive expressions for p_{ij} and Q_{ij}.

Encounter probability p_{ij}

With no knowledge of the swimming pattern, we simply assume that the fish moves approximately horizontally and at random within V_j^c. Along one of the legs the fish can only encounter a net if both the leg and the net lie within the intersection V_{ij}^{cs} of V_j^c and V_i^s. The probability of this is $V_{ij}^{cs}/V_j^c \cdot V_{ij}^{cs}/V_i^s$. If this is the case, the probability per net area and per swimming distance of actually encountering the net is found by averaging over the angle between the leg and the net, giving $(2/\pi)V_{ij}^{cs-1}$. Multiplying these two probabilities, we get

$$(4) \quad p_{ij} = \frac{2}{\pi} \frac{V^{cs}}{V_j^c V_i^s}.$$

Retention probability Q_{ij}

In the analysis of selectivity curves, several authors make assumptions about the geometrical relationship between the size of the fish and the mesh (see Hamley 1975). The hypothesis of geometrical similarity states that the maximum girth G of the most efficiently caught fish (the modal girth) is proportional to the mesh perimeter $M = 4m$, or $G = \kappa M$ (see Hamley 1975).

Stated in this way, the hypothesis of geometrical similarity refers to the girth having the maximum selectivity for a given mesh. However, the selectivity depends on both encounter probability and retention probability, and only the latter is related to the geometry of fish and mesh. Therefore, a stronger geometrical relationship could be valid for the retention probabilities under approximately identical external conditions.

Consider the variation of the retention probability $Q(m,g)$ with m for an arbitrary, fixed g. We denote the corresponding curve a QB curve. We propose the following global geometrical similarity hypothesis for $Q(m,g)$:

$$(5) \quad Q(\alpha m,\alpha g) = f(\alpha,g)Q(m,g).$$

If m and g are increased by the same factor α, Q is invariant, except for a scaling factor $f(\alpha,g)$ which is constant for each QB curve and expresses the effect of scaling on the height of the QB curves. Eq. (5) implies that

$$(6) \quad Q(m,g) = (g/g_0)^s F(m/g).$$

The scaling ratio α can be expressed by g/g_0, where g_0 is some chosen reference girth. F can be chosen at will, and s = constant is the scaling exponent.

The maximum $Q_B(g)$ of $Q(m, g)$ for a fixed g is obtained for a fixed ratio m/g, and $Q_B(\alpha g)/Q_B(g) = \alpha^s$. Similar results are obtained for the QA curves giving Q as a function of g for a given mesh m.

A convenient choice for the function F was

$$(7) \quad F\left(\frac{m}{g}\right) = q \exp\left(\frac{p}{r}\right)\left(\frac{m}{\theta g}\right)^p \exp\left(\frac{p}{r}\left(\frac{m}{\theta g}\right)^r\right)$$

where θ, p, q, and r are constants ≥ 0. All moments of Q with respect to both m and g can then be calculated in terms of gamma functions. F is skewed one way or the other, only depending on p and r. Its global maximum is obtained at $m/g = \theta$, and $F(\theta) = q$, $Q_B(g) = q(g/g_0)^s$. Accordingly, q is the maximum value of the QB curve for the reference girth g_0.

With this explicit form for F the maximum $Q_A(m)$ of the QA curves is obtained for $g = (m/\theta)(1 - s/p)^{-1/r}$. If s is small and p and r are large, $g \approx m/\theta$ and $Q_A(m) \approx q(m/ag_0)^s$.

Finally, as not all fish are caught at the maximum girth, we assume that g is an efficient girth related to the maximum girth G by $g = G - G_0$. With these assumptions, $Q_{ij} = Q(m_i, g_j)$.

Capture probability P_{ij}

At the end of each leg covered, the fish will be in one of the following $I + 1$ states:

State i, $i = 1, \ldots, I$: the fish is retained in a net of type i.
State $I + 1$: the fish is still free.

The nonzero elements of the transfer matrix T giving the probability of transfer from one state to an other during the passage of one leg are then

$$T_{i,i} = 1$$

$$T_{i,I+1} = p_{ij} E_i Q_{ij} D_j/R$$

$$T_{I+1,I+1} = 1 - D_j Z_j/R$$

where

$$Z_j = \sum_i p_{ij} E_i Q_{ij}.$$

The probability P_{ij} that the fish is captured in one of the nets of type i after all R legs is the ith component in $T^R V_0$, where $V_0 = [0, 0, \ldots, 0, 1]'$ is the initial state vector. When $R \to \infty$:

$$(8) \quad P_{ij} = \frac{p_{ij} E_i Q_{ij}}{Z_j}(1 - e^{-D_j Z_j}).$$

The total capture probability is $P_j = 1 - \exp(-D_j Z_j)$. The capture probability in nets of type i is proportional to the total capture probability and to the relative contribution of these nets to Z_j. If $D_j Z_j \ll 1$, $P_j \approx D_j Z_j$, and Eq. (8) simplifies to Eq. (3) with P_{ij} given by Eq. (4).

As $P_{ij} = C_{ij}/N_j$, comparison with Eq. (2) gives

$$(9) \quad S_{ij} = \frac{p_{ij} Q_{ij}}{Z_j}(1 - e^{-D_j Z_j}).$$

Application of the Model

The model was used to estimate Q_{ij} and D_{jk} from the observed catch data and the population estimates. Since the catches during one night in all cases were small compared with the total population, and nets of a given mesh size were never set more than once in each location during each season, we assumed that the total catch in each season would have been the same if the same fishing effort had been concentrated in a single night. Accordingly, the total catch probability for girth-class j may be estimated by $\hat{P}_j = C_j/N_j$, and the estimate of P_{ij} is $\hat{P}_{ij} = C_{ij}^p/N_j + C_{ij}^l/N_j$.

The sampling volumes V_i^{sp} and V_i^{sl} for pelagic and littoral nets were disjoint and independent of i and covered the whole volume of the lake down to 12 m. Their subscripts i can therefore be dropped. We assume that the cruising volumes V_j^c were completely situated above this depth, i.e. we disregard any part of the path lying more than 12 m below the surface. Then, V_j^c equals the union of its intersections with V^{sp} and V^{sl}; henceforth, $V_j^c = V_j^{cp} + V_j^{cl}$. Accordingly, D_{jk} is the sum of two parts $D_{jk}^p = (V_j^{cp}/V_j^c)D_{jk}$ and $D_{jk}^p = (V_j^{cp}/V_j^c)D_{jk}$ lying in V_j^{cp} and V_j^{cl}, respectively.

Applying Eq. (8) to pelagic and littoral nets separately gives $Q_{ij} D_{jk}^p = Y_{ijk}^p$ and $Q_{ij} D_{jk}^l = Y_{ijk}^l$, where

$$(10) \quad Y_{ijk}^p = -\frac{\pi V^{sp} C_{ijk}^p}{2 E_{ik}^p C_{jk}^p} \ln\left(1 - \frac{C_{jk}^p}{N_{jk}}\right)$$

and Y_{ijk}^l is given by a similar expression. Then

$$(11) \quad Q_{ij} D_{jk} = Y_{ijk}^p + Y_{ijk}^l = Y_{ijk}.$$

To reduce the variances, we replaced Y_{ijk} in Eq. (11) by its moving average over three neighbouring j-values for fixed i and k, with weights 1/4, 1/2, and 1/4. The parameters in the model were then estimated in two steps.

Step 1: If $Y_{ijk} \neq 0$, Eq. (11) can be linearized by taking logarithms, and estimates for Q_{ij} and D_{jk} are obtained by least squares. However, if Y_{ijk} is small, its logarithm approaches minus infinity and the corresponding term will give a diverging contribution to the estimate. This problem can be avoided by weighting the terms in the least squares sum. A theoretical analysis of the variances showed that the weights Ω_{ij} should be chosen proportional to Y_{ijk}. A convenient choice is

$$(12) \quad \Omega_{ij} = \left(\prod_k Y_{ijk}\right)^{\frac{1}{K}}.$$

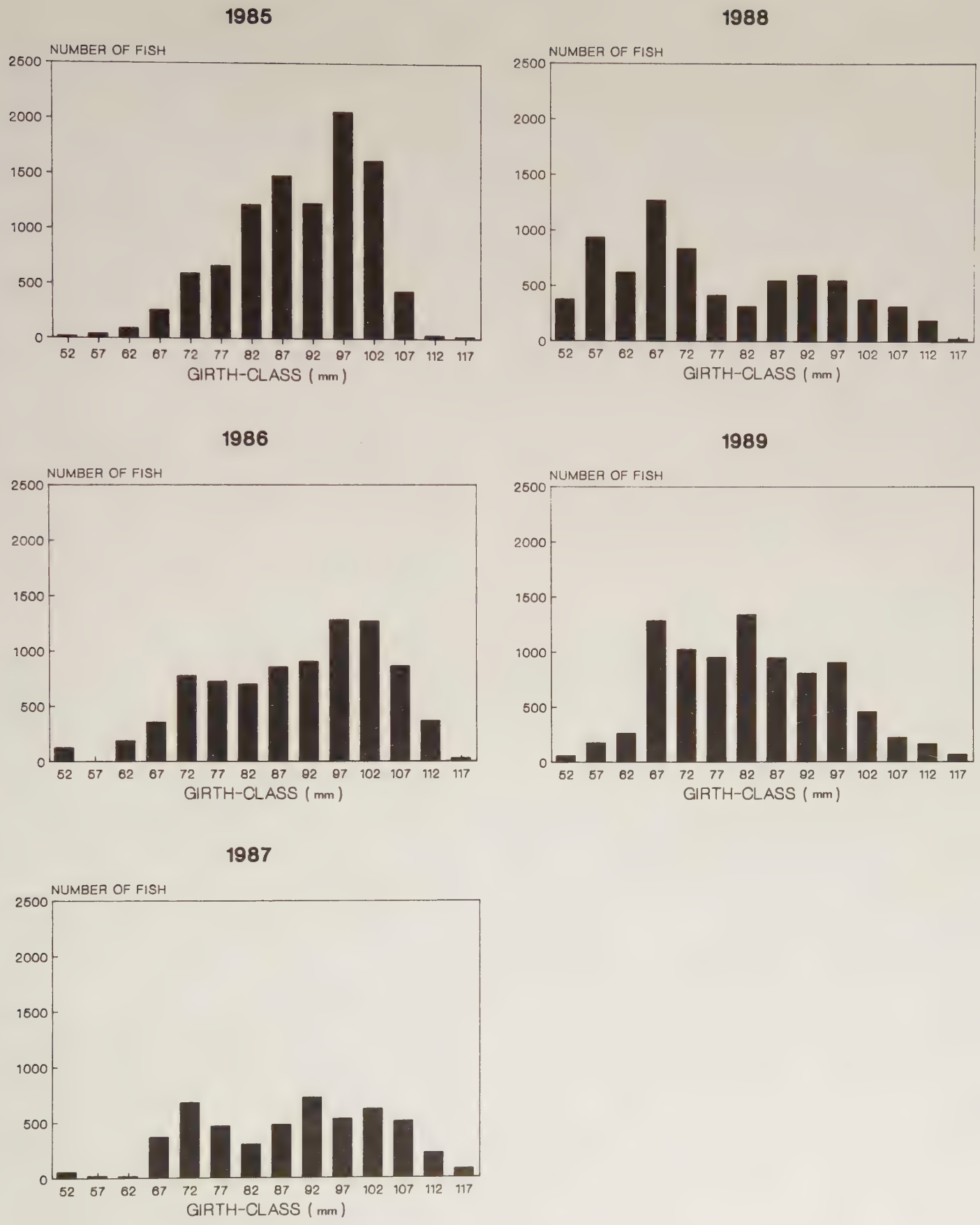

FIG. 1. Estimated number of brown trout in girth-classes 52–117 mm in Lake Løyning, June 1985 – June 1989.

Introducing

$$\omega_{ij} = \Omega_{ij}\left(\sum_{\lambda}\Omega_{\lambda j}\right)^{-1},$$

a solution is

$$(13)\quad Q_{ij} = \left(\prod_{k} Y_{ijk}\right)^{\frac{I}{K}}\left(\prod_{\lambda,\mu} Y_{\lambda j\mu}^{-\frac{1}{K}\omega_{\lambda j}}\right)$$

$$(14)\quad D_{jk} = \prod_{\lambda} Y_{\lambda jk}^{\omega_{\lambda j}}.$$

The ranges of λ and μ equal those for i and k, respectively. The solution is not unique, since Q_{ij} could be multiplied by a factor h_j and D_{jk} divided by the same factor.

Step 2: The parameters in Eqs. (6) and (7) can now be estimated by fitting Q (m_i, g_j) to the values of Q_{ij} calculated from Eq. (13) for all mesh values. Various methods have been employed to fit selectivity models to a girth–mesh response surface (Wulff 1986; Boy and Crivelli 1988; Helser et al. 1990). We used least squares and solved the minimalization problem by using the function Minerr in MathCAD (Anonymous 1989).

Results

Direct Estimation of Mesh Size Selectivities

During the sampling period 1985–89, the total number of fish within girth-classes 67–117 mm varied between 5000 and 9600 (Fig. 1). The population structure changed from a dominance of girth-classes ≥ 92 mm (90–94 mm) in 1985–86 to a dominance of girth-classes <92 mm in 1988–89. The number of fish in girth-classes 52–62 mm was below 300 in 1985–87, while in 1988 the number had increased to nearly 2000, with a decrease to 500 in 1989 (Fig. 1).

The combined catch per night by one littoral and one pelagic gill net declined with increasing mesh size, both in total number and maximum number. Except for the catch by mesh size 16.0 mm, which in all years appeared with high and rather similar values for girth-classes 67–77 mm, catches showed a declining trend from 1985–86 to 1987–88, with a small increase in 1989.

The selectivities of each mesh size, estimated directly according to Eq. (2), varied annually (Fig. 2). The height of the selectivity curves decreased from mesh size 16.0 to 21.0 mm and were approximately equal for mesh sizes 21.0–26.0 mm. The modal girth-class (i.e. the girth-class with maximum selectivity for a given mesh size) increased with increasing mesh size from girth-class 67 mm in mesh size 16.0 mm to girth-class 112 mm in mesh size 26.0 mm (Fig. 2).

Estimated Retention Probabilities

Outside the girth interval 82–112 mm, comprising seven girth-classes, the data were too sparse to allow any reliable fit. The percentages of all fish in these girth-classes in the five years were 84, 84, 76, 43, and 60%, respectively. To estimate Q_{ij}, we used two data sets: all five years (1985–89) and five mesh sizes (excluding 22.5 mm) and four years (1986–89) and all six mesh sizes.

TABLE 1. Estimated values for the parameters of the retention probability function in Eqs. (6) and (7), based on the Q_{ij} values calculated from Eq. (13). The chosen reference girth g_0 = 82 mm.

Data set	θ	G_0	p	q	r	s
5 years	0.271	17.1	5.02	1.50	11.6	−0.26
4 years	0.266	16.2	7.35	1.71	10.6	−0.70

A fit supporting the global geometrical similarity hypothesis was obtained for both data sets (Table 1). The residual sum of squares in the two cases was 1.21 and 1.14, respectively. However, some Q values exceed 1. A global normalization can be performed by introducing h_j = constant in Eq. (13). The normalization has been chosen such that $Q_B(g_0) = 1$. Formally this is done by introducing $h_j = 1/q$ in Eqs. (13) and (14), in practice by dividing all Q values by q and multiplying the swimming distances as given by Eq (14) by q. With this normalization, the QB curves are uniquely determined from the fit (Fig. 3). For both data sets the scaling effect is small, resulting in a slight decrease in $Q_B(g)$ with increasing girth. The shape of the QA curves depends on the scaling exponent s (Fig. 4). The displacement of the maximum of both QA and QB curves and the decrease in maximum retention probability with increasing girth or mesh are demonstrated in Fig. 5.

Using the average of the two values for θ, we get $g = (1/4\theta)M = 0.93\,M$ at the maximum of the QB curves. The ratio of maximum girth $G = g + G_0$ to M varied from 1.09 (for m = 26 mm) to 1.20 (m = 16 mm).

Swimming Distances and Encounter Probabilities

In all five years the swimming distances are in general decreasing with increasing girth, with a levelling out for the larger girth-classes. The only exception is 1988, when the distances increased with increasing girth for the higher girth-classes (Fig. 6). There are large annual variations within each girth-class (Fig. 7). Except for the year 1988, all curves behave in roughly the same fashion. In 1988 the curves seem more perturbed, in particular for girth-classes 107 and 112 mm.

The annual variation of the estimated swimming distances for the seven girth-classes is independent of the scaling exponent s. However, a different value of s would change the relative height of the curves.

In the simplified situation when Eq. (3) is valid, the encounter probabilities can be calculated from Eqs. (3) and (4).

Discussion

Because we obtained a satisfactory global fit to the retention probability values for both data sets, with approximately equal values for the parameters, we conclude that the geometrical similarity hypothesis is confirmed. If the assumptions behind the model are valid, the obtained model for the retention probabilities Q will have a general validity for brown trout within the studied girth range.

Rudstam et al. (1984) assumed an equal probability of retention for the modal fish encountering gill nets. The maximum of the QB curve is, however, not necessarily independent of girth, even if the geometrical similarity hypothesis is valid, and may depend on fish size in a variety of ways. Older and larger fish could more easily detect the nets, since visual acuity improves with fish size (Hester 1968), and also be more able to avoid the nets. Nets with a large mesh size are less visible

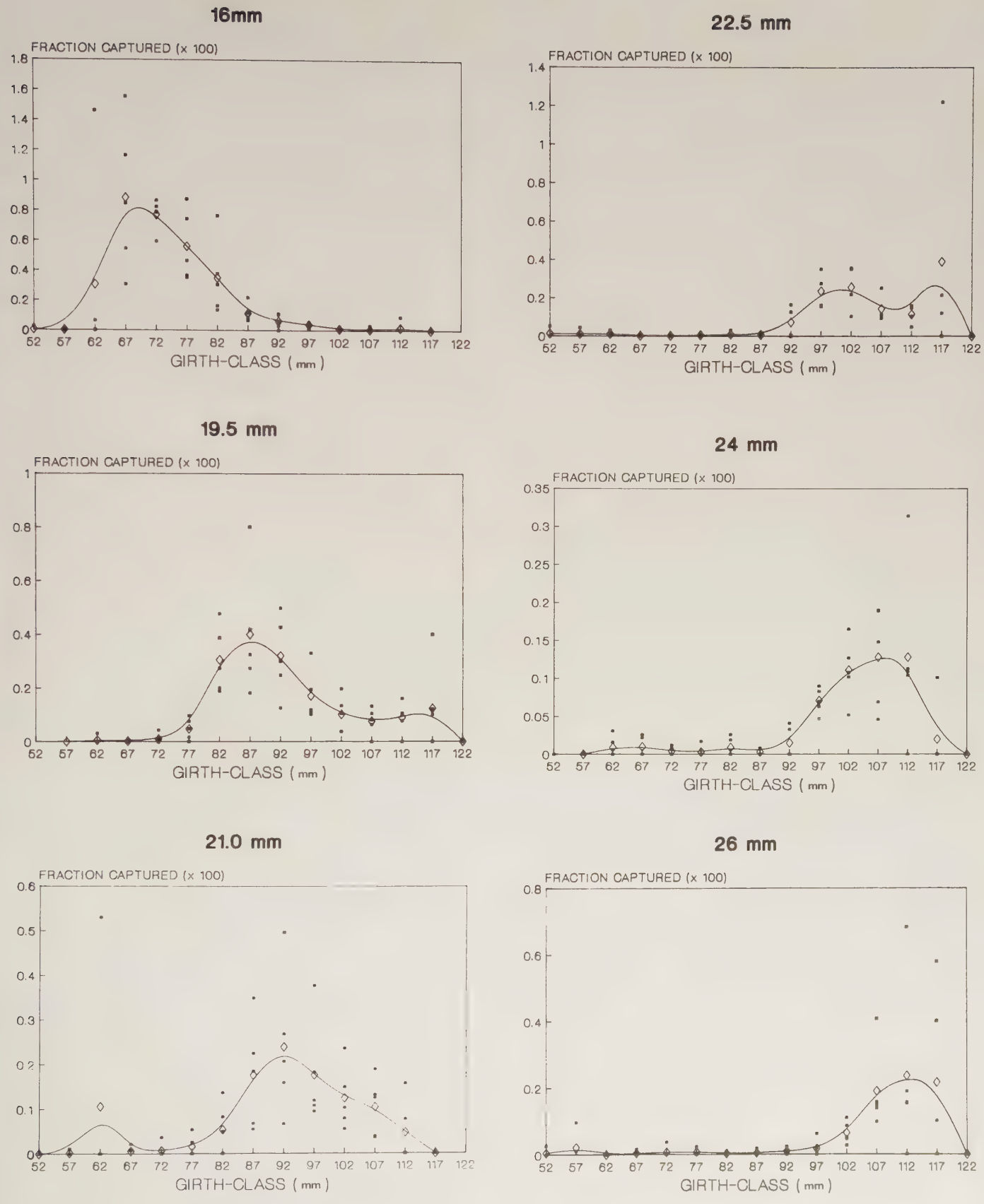

FIG. 2. Estimated fraction of each girth-class captured per night by one littoral and one pelagic gill net with mesh sizes from 16.0 to 26 mm (knot to knot measure). Note the different vertical scales. Squares = annual observations; diamonds = mean values.

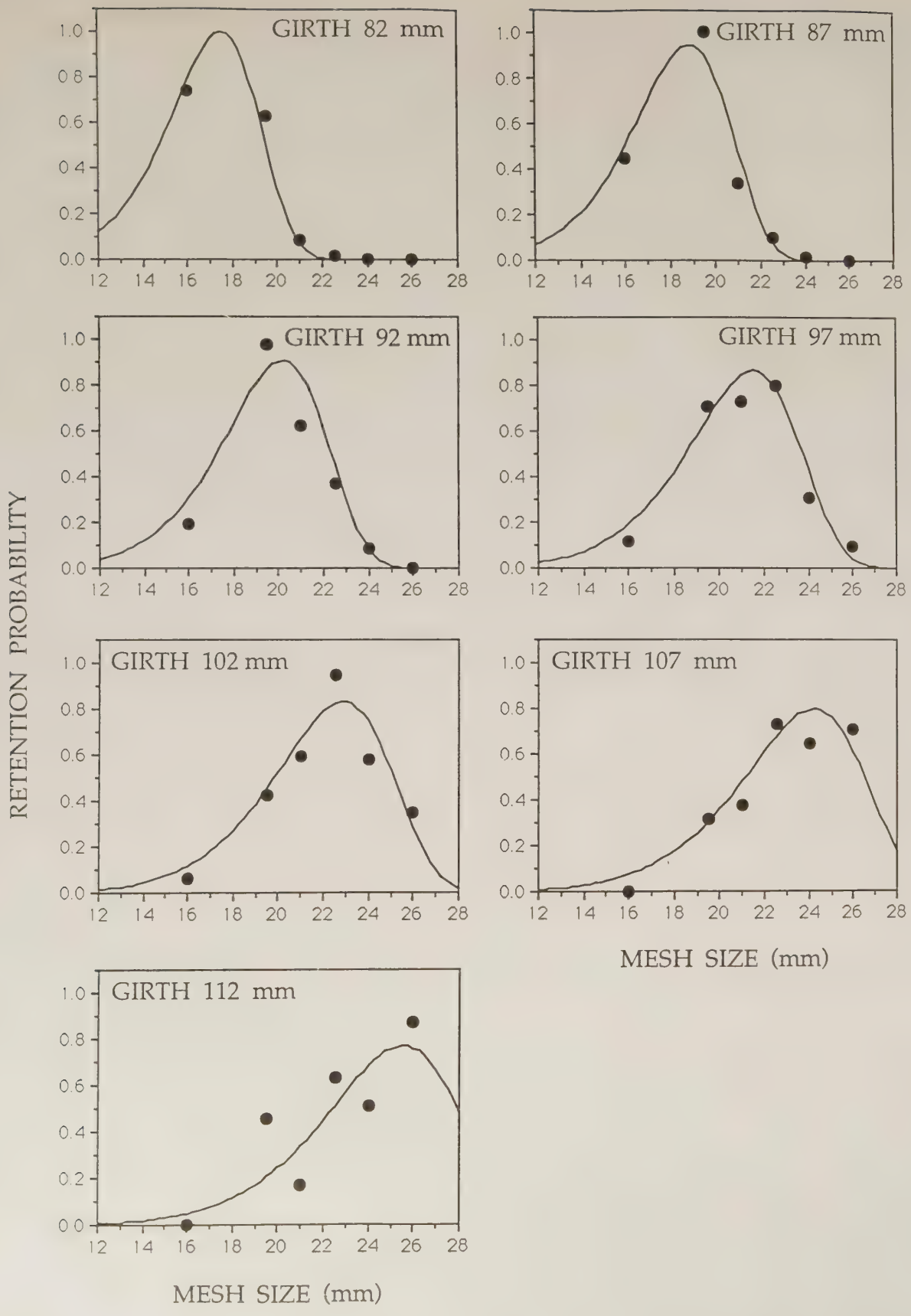

FIG. 3. Probability of retention as a function of mesh size for the seven girth-classes 82–112 mm (*QB* curves). The curves represent the function given by Eqs. (6) and (7), fitted to Q_{ij} values (circles) estimated from the 1986–89 data. Both $Q(m,g)$ and Q_{ij} are normalized to give $Q_B(g) = 1$ for $g = 82$ mm.

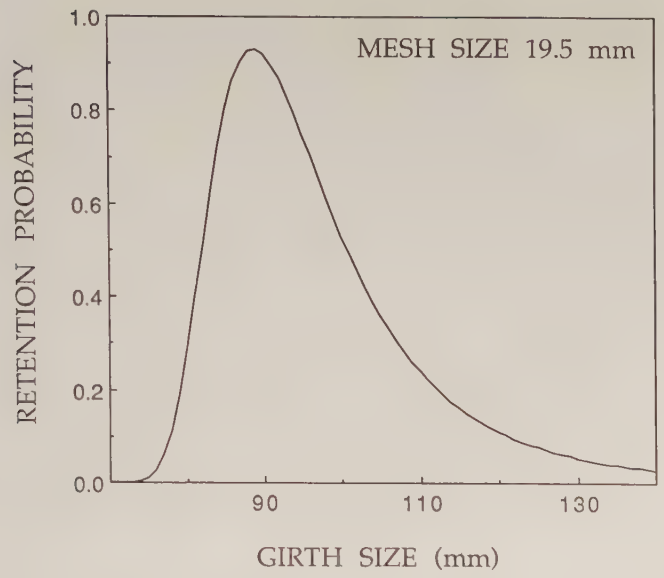

FIG. 4. Retention probability $Q(m,g)$ as a function of girth for mesh size 19.5 mm. Parameter values and normalization are the same as in Fig. 3.

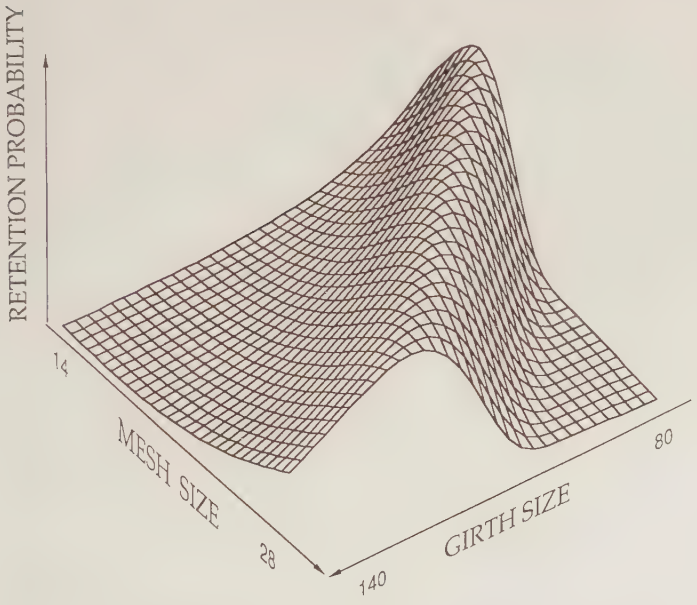

FIG. 5. Three-dimensional plot of the $Q(m,g)$ surface in arbitrary vertical units. The g-axis points to the left towards the reader and the m-axis to the right. The girth range is from 80 to 140 mm and the mesh range from 14 to 28 mm.

than finer nets, but the difference is small (Hester and Taylor 1965). On the other hand, unless larger fish have lower swimming speed than smaller fish, their momentum and turning radius will be larger. In the case of physical contact with the net, a heavy fish will produce a deeper bag in the net and get entangled more easily. We found a small negative scaling effect, but the scaling exponent s is not uniquely determined and could be modified by introducing other values for h_j in Eq. (14). However, we do attach some weight to the fact that a good fit was obtained without any recourse to additional ad hoc modifications of the h_j.

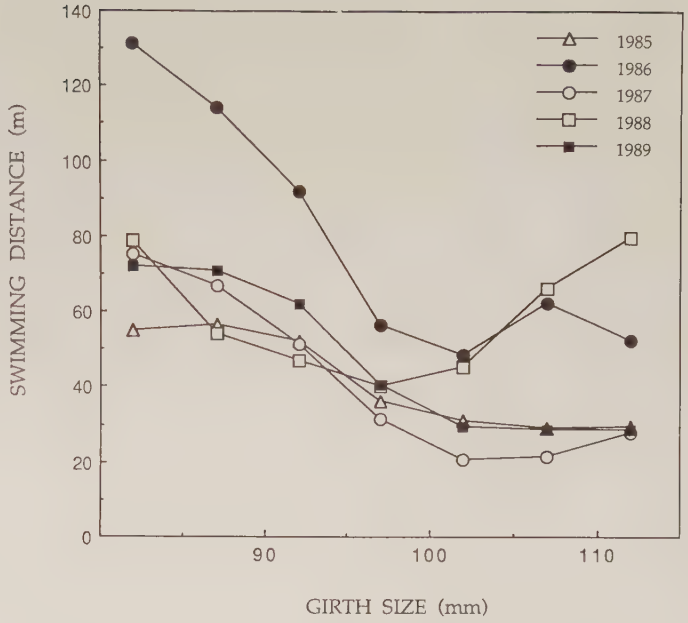

FIG. 6. Swimming distances as a function of girth for each of the five years 1985–89. The points represent the distances calculated from Eq. (14) with the same normalization as in Fig. 3 and 4.

Several authors assume proportionality between maximum girth and mesh size at the maximum of the selectivity curve (Hamley 1975). In the present study, an effective girth is proportional to the mesh, and the reason that $g/M < 1$ is explained by the fact that fish too small to be wedged also were tangled or gilled. The estimated ratio $G = g + G_0$ to M could be compared with the ratios 1.08–1.35 of G to M quoted by Hamley (1975), but one should keep in mind that the latter refer to selectivity curves.

The decreasing trend in selectivity values with increasing mesh size documented in this study is contrary to expectations based on earlier results, where the gillnet selectivity for several species increased with increasing mesh size and fish size (e.g. Hamley and Regier 1973; Hamley 1980; Rudstam et al. 1984; Borgstrøm 1989; Winters and Wheeler 1990). As the retention probability at most can be expected to vary slightly with girth within the observed girth range, the decrease in selectivity is likely to be explained by decreasing swimming distances. From our model the estimated horizontal swimming distances in a given year decreased with ratios of 2–3 from the smallest girth-classes to higher, which is much too large to be explained by variations in the maximum retention probabilities. If the maxima of the QB curves were all equal, this ratio would have been even larger.

Taxonomically and ecologically related species behave differently when searching for prey: while, for instance, whitefish move continuously without resting, brown trout make short excursions to catch suitable prey (Nilsson 1978). For a given species, swimming pattern may also differ with fish size, as shown, for example, by Rudstam et al. (1984). The swimming distances, and accordingly also the selectivities, are functions of many factors, among which fish behaviour, population density, and food availability probably are the more important. We tried in various ways to relate the distance variations to fish abundance and water temperature but were unable to find any convincing correlations.

An explanation of the decrease in estimated swimming distances could be sought in the varying ratio between energy

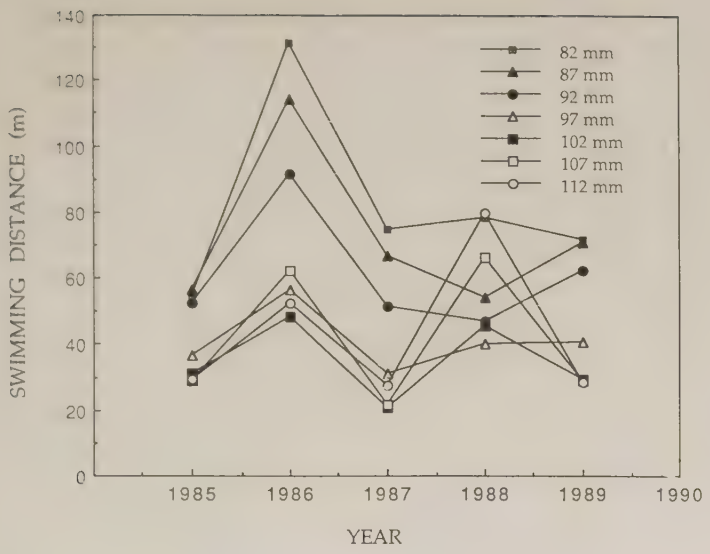

FIG. 7. Estimated annual variations in swimming distances for each of the seven girth classes 82–112 mm. The points are the same as in Fig. 6.

return per swimming distance and fish size. During the first 4 yr of life the average annual length increment of brown trout in Lake Løyning was 4–5 cm, but after the first spawning the growth in length stagnates (Borgstrøm and Heggenes 1990), indicating food shortage. The energy demand increases strongly with fish size at summer temperatures, while the prey encounter rate at low prey densities is only slightly greater for larger fish than for smaller fish (Hamrin and Persson 1986). The optimal swimming distance may then be longer for small fish than for larger fish.

When variations in swimming distance are not taken into consideration, as has been the rule in most indirect estimations of gillnet selectivity (e.g. Jensen 1977; Jensen 1986; Boy and Crivelli 1988; Helser et al. 1990), serious errors in the assessment of population size structure may arise. Even when gillnet selectivities have been previously estimated directly, annual variations in fish behaviour may give a biased estimate of size structure (Fig. 7). These arguments point to some inherent limitations in gillnetting as a method to estimate population abundances and size structures.

Our model is based on observable variables related to fish behaviour and net properties. Some variables, such as swimming distance, for example, are largely related to the behaviour of the fish and vary with fish size and the specific conditions in the lake studied. Other variables, such as retention probability for a given fish size, for example, are specific to the net and have probably an approximate validity independent of the lake studied. Thus, it seems to be an advantage to separate these two classes of variables in models of gillnet selectivities. We believe that this approach makes our model more robust and better suited to making predictions than models where variables and parameters have less realistic significance. Moreover, it is easier to check and could be related to results from other experiments on fish behaviour.

By supplementing the catch data with direct observations of the swimming pattern, or of the catch and retention process for fish from different size-classes, estimates for the two undetermined parameters q and s in Eqs. (6) and (7) could be obtained. Then the absolute swimming distances and the absolute retention probabilities for all size-classes and all meshes would be known. This indicates that future research on gillnet selectivities should to a much larger extent be based on an understanding of fish behaviour and its dependence on external factors. A future goal might be to be able to predict the size of an unknown population from net catches and observed swimming behaviour, using generally valid expressions for retention probabilities for the species in consideration.

Acknowledgements

We thank T. Reisæther, T. Rønhovde, O. Lægreid, A. Borgstrøm, and R. Borgstrøm for technical field assistance and I. Helland for valuable advice on statistical problems. The study has been partly financed by Røldal Fishery Fund and the Norwegian Research Council for Science and the Humanities' research programme "Dynamics and enhancement of freshwater fish populations." We also acknowledge L. Hjeljord for correcting the English and J. Heggenes for valuable criticism of the manuscript.

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Optimizing Sampling from Trawl Catches: Contemporaneous Multistage Sampling for Age and Length structures

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Horppila, J., and H. Peltonen. 1992. Optimizing sampling from trawl catches: contemporaneous multistage sampling for age and length structures. Can. J. Fish. Aquat. Sci. 49: 1555–1559.

The objective of this study was to find out the optimal sampling strategy for contemporaneous multistage sampling of age and length structures of trawl catches. Samples were taken from a roach (*Rutilus rutilus*) stock of Lake Vesijärvi, southern Finland. Two-stage sampling proved to be superior to three-stage sampling (i.e. subsamples from the trawl catches are unnecessary). Assuming that all the age and length groups are equally important, the optimal strategy is to sample 34 fish from each of 46 loads. Compared with the present scheme (500 fish from each of 10 loads), this design cuts down the total variance of the estimated proportions of different age and length groups to about one third. An age–length key was used when estimating the proportions of the age groups. The benefits of the applied age–length key were obvious. The estimates of the proportions of different age groups were more accurate than those obtained with age readings only. Increasing the fraction of age-determined fish increases the costs, diminishes the optimal number of samples, and consequently increases the variance of the estimated proportions of different groups.

On a tenté d'établir la stratégie optimale d'échantillonnage en plusieurs étapes en vue de déterminer la structure des âges et des longueurs dans les prises au chalut. Les échantillons ont été prélevés d'un stock de gardon (*Rutilus rutilus*) du lac Vesijärvi, situé dans le secteur sud de la Finlande. L'échantillonnage en deux étapes s'est révélé supérieur à l'échantillonnage en trois étapes (c'est-à-dire qu'il n'est pas nécessaire de prélever des sous-échantillons des prises au chalut). Si l'on suppose que tous les groupes d'âge et de longueur sont également importants, l'échantillonnage de 34 poissons dans chacune de 46 charges constitue la stratégie optimale. Par rapport au schème d'échantillonnage courant, soit 500 poissons dans chacune de 10 charges, le présent plan réduit la variance totale des pourcentages estimés des différents groupes d'âge et de longueur à environ un tiers. On s'est servi d'une clé âge–longueur pour établir le pourcentage des groupes d'âge. Les avantages d'une telle clé étaient évidents. Ainsi, les estimations des pourcentages des différents groupes d'âge étaient plus précises que celles obtenues par lecture des âges. Une augmentation du nombre échantillonné de poissons d'âge déterminé mène à une augmentation des coûts, une baisse du nombre optimal d'échantillons et, par conséquent, à une augmentation de la variance du pourcentage estimatif des différents groupes.

Received April 8, 1991
Accepted February 7, 1992
(JA982)

Reçu le 8 avril 1991
Accepté le 7 février 1992

The importance of well-planned sampling programmes in fisheries research is often underestimated, especially in freshwaters. Some authors (e.g. Ketchen 1950; Schweigert and Sibert 1982) have published methods for multistage sampling in fisheries research, but in practice these methods are not always used. Additionally, most of the published sampling strategies have dealt with the age structure of fish stocks. Although age structure is one of the basic sources of information in fish stock assessment, from the biological point of view the length distribution is in some cases a more reasonable subject for research. It is possible that sampling resources should be allocated differently if length structure is also taken into consideration.

The material for this study was collected from roach (*Rutilus rutilus*) catches in 1990 from Lake Vesijärvi, southern Finland. Fishing with trawls in the Enonselkä basin (26 km^2, mean depth 6.8 m) at the southern end of the lake has been started to reduce the negative effects of the very dense roach and smelt (*Osmerus eperlanus*) stocks on the water quality and to restore the lake by biomanipulation. It has been observed that trawl fishing effectively removes roaches from the trawling area (Peltonen and Horppila 1992). The Enonselkä basin can be considered as a separate lake, since only narrow straits connect it with other parts of the lake.

Lake Vesijärvi was eutrophicated by the City of Lahti during the 1960's and early 1970's. The municipal sewage loading was diverted from the lake in 1976 and the lake started to recover. However, in the 1980's, blue-green algal blooms increased again and the recovery of the lake faded. Enclosure experiments carried out in the lake demonstrated that high roach biomass is one of the key factors maintaining high algal productivity and biomass (Horppila and Kairesalo 1990).

Roaches can change the water quality by size-selective predation on zooplankton, by bioturbation, and by their excretory products (Andersson et al. 1978; Henrikson et al. 1980; Brabrand et al. 1990; Horppila and Kairesalo 1990). In the enclosure experiments carried out in Lake Vesijärvi, bioturbation of sediments and recycling of nutrients, rather than zooplanktivory, seemed to be the main factors linking roaches to water quality. Roaches have omnivorous feeding habits; they

are planktivorous as juveniles, but become more benthivorous as adults (Niederholzer and Hofer 1980; Brabrand 1985; Jamet et al. 1990). Since the diet of an individual fish is determined by its size rather than by its age, the length structure of the stock is of great importance to a study which investigates the contribution of the roach stock on the quality of the water. On the other hand, in stock assessment the age structure is of primary importance. In this study, we are trying to find out the optimum strategy for contemporaneous sampling for length and age structures of the roach catches in the Enonselkä basin and to compare the 1990 sampling with an optimal scheme. Additionally, the benefits of the applied age–length key are studied.

Materials and Methods

In 1990, trawling took place from May to August. The roach catch was about 87 metric tons (48% of the total catch). Samples were taken from the landed catches 10 times during the summer. Each sample included five randomly selected subsamples of about 100 roaches. The total length of each fish was measured. Additionally, four times (May 29, June 18, July 23, and August 21) during the sampling period, about 100 roaches were selected for age determination. Using the age-determined samples and a derived age–length key (Ketchen 1950), the length distributions were used to estimate age distributions.

To estimate the proportions, variance components, and optimal number of sampling units, we used the equations of Cochran (1977) as in Schweigert and Sibert (1983).

The variance of the mean proportion of fish at age or length group L can be estimated with equation

$$(1) \quad V(P_{L...}) = \frac{S_1^2}{n} + \frac{S_2^2}{nm} + \frac{S_3^2}{nmk}$$

where n is the number of loads sampled, m is the number of samples from a load, and k is the number of fish at age or length group whose ages were determined or whose lengths were measured.

The variance component caused by the among-load variation is given by

$$(2) \quad S_1^2 = \sum_{i=1}^{n} \frac{(P_{Li..} - P_{L...})^2}{n-1}$$

where $P_{Li..}$ is the mean proportion of fish at age or length group L in load i and $P_{L...}$ is the mean proportion of fish at age or length group L in the catch.

The variance component due to among-sample variation is given by

$$(3) \quad S_2^2 = \sum_{i=1}^{n} \sum_{j=1}^{m} \frac{(P_{Lij.} - P_{Li..})^2}{n(m-1)}$$

where $P_{Lij.}$ is the mean proportion of fish at age or length group L in sample j of load i.

The variance component caused by among-fish variation is given by

$$(4) \quad S_3^2 = \frac{k}{nm(k-1)} \sum_{i=1}^{n} \sum_{j=1}^{m} P_{Lij.}(1 - P_{Lij.}).$$

If the cost of a survey is considered, the equation

$$(5) \quad m' = \sqrt{\frac{c_1 S_2^2}{c_2 S_1^2}}$$

gives the value of m, which minimizes the product of variance and cost. In the equation, c_1 is the cost of obtaining a load and c_2 is the cost of obtaining a sample from a load.

If the value of m' for all the age or length groups is near 1, two-stage sampling can be used. In this situation, the estimate of variance is given by

$$(6) \quad V(P_{L..}) = \frac{S_1^2}{n} + \frac{S_3^2}{nk}$$

where n is the number of the loads sampled and k is the number of fish sampled per load.

The variance component due to among-load variation is given by

$$(7) \quad S_1^2 = \sum_{i=1}^{n} \frac{(P_{Li.} - P_{L..})^2}{n-1}$$

where $P_{L..}$ is the estimated mean proportion at age or length L in the catch and $P_{Li.}$ is the mean proportion at age or length L in sample i.

The variance component caused by among-fish variation is

$$(8) \quad S_3^2 = \frac{k}{n(k-1)} \sum_{i=1}^{n} P_{Li.}(1 - P_{Li.}).$$

The total cost (C) of the sampling program is given by

$$(9) \quad C = c_1 n + c_2 nk + c_3 nmk$$

for three-stage sampling and by

$$(10) \quad C = c_1 n + c_3 nk$$

for two-stage sampling where c_3 is the cost of determining the age or length of a fish.

Because the financial costs of taking a sample, determining ages of fish, etc., are dependent on the time used, we used time instead of money to describe the cost structure of the study. It was approximated that it takes 5 h to sample a load (c_1), 6 min to obtain a subsample from a load (c_2), and 3 min to determine the age of a fish (c_3). The value of c_3 is low because the age–length key was used to determine ages. Total sampling time was constrained to 305 h, the time used to sample in 1990, to facilitate comparisons between the 1990 sampling and the optimal scheme.

Results

The values of m' (optimal number of subsamples) indicate whether three-stage sampling was worthwhile. It seems clear that two-stage sampling is appropriate for the age structure of the catch (average $m' = 1.1$). Only age group 3 would require three-stage sampling ($m' = 2.0$).

Length intervals of 4 cm (Brabrand 1985) and 5 cm (Rask 1989) have been found appropriate when studying the diet of roaches. Consequently, we decided to apply the 4-cm intervals, and two-stage sampling turned out to be appropriate (average $m' = 1.3$). Only the smallest (<100 mm) fish require three-stage sampling ($m' = 2.5$). We also examined whether the sampling design is dependent on the class width applied. If 1- or 2-cm intervals were used, most length groups would require three-stage sampling (the mean values of m' are 2.2 and 2.9, respectively). Widening the class width to 3 cm causes a distinct decrease in the values of m' (average 1.5), but the marginal groups still require three-stage sampling.

For all the age and length groups the among-load variation is considerably lower than the among-fish variation (Tables 1 and 2).

TABLE 1. Estimated variance components for the age groups.

Age (yr)	S_1^2 ($\times 10^3$)	S_3^2 ($\times 10^3$)
2	20.745	74.236
3	0.738	37.923
4	2.337	66.919
5	4.885	157.135
6	8.379	171.573
7	3.265	109.673
8	4.378	109.347
9	2.137	41.919
>9	1.278	39.142

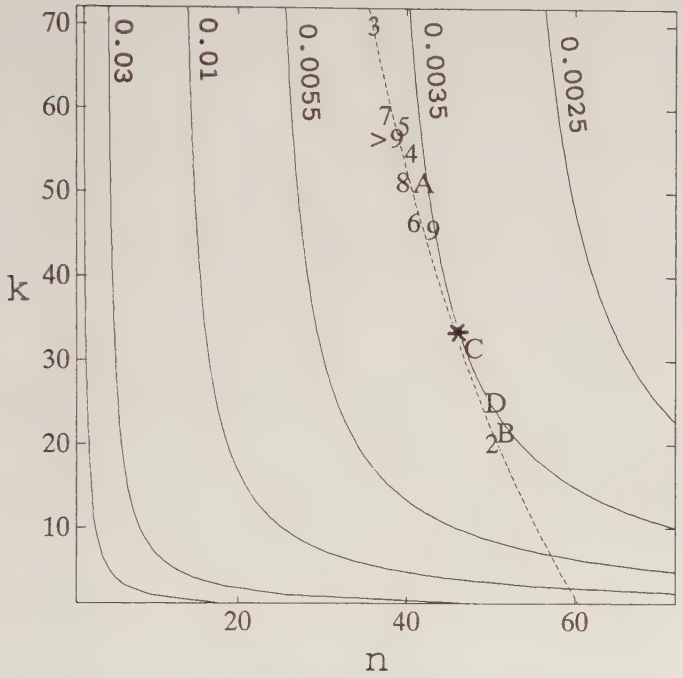

FIG. 1. Contours showing the summed variances (Eq. 6) of the proportions of all the age and length groups with different combinations of k and n. The resource curve (broken), $k\ (C - c_1 n)\ (c_3 n)^{-1}$ (see Eq. 10), shows the upper limit of sampling. Numbers 2–9 (age groups) and letters A–D (length groups <100, 100–139, 140–179, and >179 mm, respectively) on the resource curve show the optimal sampling designs for each group. The optimal scheme considering all the age and length groups is marked with an asterik.

Since all the age and length groups are considered to be equally important, the optimal sampling design minimizes the total variance summed over all the age and length groups. The levels of total variance obtained with different combinations of k and n can be presented as contours (Fig. 1). The resources restrict the range of possible values of k and n to the left-hand side of the marked curve. The best sampling design can be determined graphically: on the optimum point ($n = 46$, $k = 34$) the resource curve touches the variance surface at the lowest level (~0.0035).

The sampling carried out in 1990 ($n = 10$, $k = 500$) is far from the optimal scheme. Using these values of n and k and summing all the variances from the separate age and length groups gives the total variance of 0.0126. With the resources available the optimal sampling design lowers the total variance of the estimated proportions of the different age and length groups to about one third. The present sampling design ($n = 10$, $k = 500$) does not lie on the resource curve because a large proportion of available resources was consumed in obtaining the unnecessary subsamples.

TABLE 2. Estimated variance components for the length groups.

Length (mm)	S_1^2 ($\times 10^3$)	S_3^2 ($\times 10^3$)
<100	0.390	0.713
100–139	21.878	8.694
140–179	23.615	21.503
>179	29.143	16.053

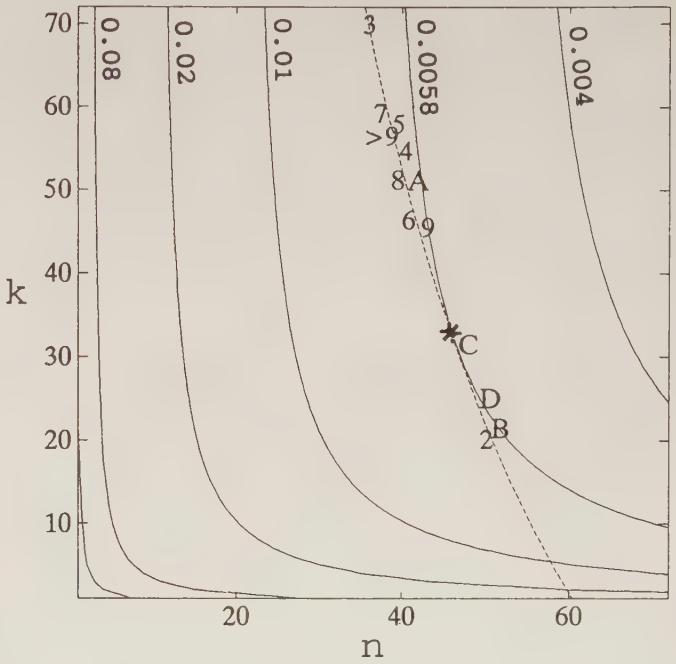

FIG. 2. Contours showing the summed weighted (Eq. 11) variances of the proportions of all the age and length groups with different combinations of k and n. The resource curve and the optimal sampling designs of each group are plotted as in Fig. 1. The optimal scheme is marked with an asterisk.

Discussion

Optimal sampling programmes can be quite different for different age groups or different length groups (Fig. 1). For instance, the optimal k for age group 2 is lower (19) and the optimal n higher (51) than for any other age group, while for age group 3 the optimal value of k (70) is highest and the value of n (36) lowest of all the groups. If some age or length group is especially important, sampling can be directed towards its optimum value with the consequence of reduced precision in estimating the proportion of some other age and length group.

One way to consider the importance of different groups is to weight the variance of each group by frequency (i.e. to consider each fish to be equally important):

$$(11)\quad V = \frac{\sum_{i=1}^{n_g} n_{Li} V_{Li}}{\Sigma 2 n_i} n_g$$

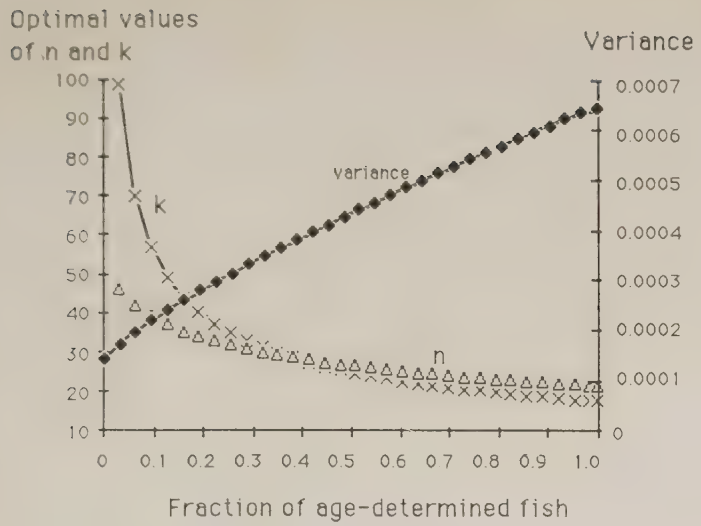

FIG. 3. Effects of the fraction of age-determined fish on the optimal values of k and n and on the variance of the estimated proportion of age group 5.

where n_{Li} is the number of fish in age or length group i, V_{Li} is the variance of each group (Eq. 6), and n_g is the number of groups (13 in this case).

Since the variances of the most abundant groups were greatest, weighting by frequencies increased the estimated total variance (Fig. 2). However, the optimal values of n and k did not change. The influence of weighting on the optimal sampling design could be significant if the catchabilities of the age and length groups were more variable.

The results (and conclusions) are quite sensitive to the choice of c_1, c_2, and c_3. The cost structure used in this paper is based on experiences gained from sampling in 1990. The exact cost structure is, however, very difficult to describe. For instance, in practice the age of a 2-yr-old fish is easier to determine than the age of a 9-yr-old fish, but it would be too complicated to consider this in the calculations.

It is not possible for us to affect c_1 (the cost of obtaining a load), but c_3 (the cost of determining the age of a fish) can be affected by changing the proportion of the age-determined fish when the age–length key is applied. Increasing the fraction of age-determined fish increases c_3 and consequently diminishes the optimum values of k and n until the fraction of age-determined fish reaches 20% (Fig. 3). Beyond 20% the proportion of age-determined fish does not remarkably affect the values of k and n. Nevertheless, when the proportion of age-determined fish is increased, the variance of the estimated proportion of an age group increases continuously. If unbiased estimates can be obtained with the age–length key, the fraction of age-determined fish should be small because of the continuous increase in variance.

The variance estimates obtained with the age–length key should be smaller than those obtained with age readings only, so as not to cause additional error in the estimate of total variance in an age group. The benefits of applying the age–length key were explored with the equations of Kimura (1977). We compared the variance estimates of the age groups in separate catches obtained with the age–length key with the respective variance estimates if only age determinations were used. The variance of the proportion of an age group when estimated with the age–length key (if a fixed number of fish is aged per sample) is given by

TABLE 3. Estimated variances ($\times 10^3$) of the proportions of age groups 2, 5, and 9 when the age–length key is applied (A–L) and when only age determinations are used (A).

	Age (yr)					
	2		5		9	
Load	A-L	A	A-L	A	A-L	A
1	2.680	0.104	2.390	5.054	0.053	0.083
2	0.217	3.701	1.857	3.480	0.240	0.606
3	0.058	2.612	2.310	4.367	0.317	0.247
4	0.097	2.216	2.114	3.656	0.440	0.742
5	0.017	0.368	2.117	2.898	0.432	0.723
6	0.138	0.971	4.537	2.685	0.003	0.838
7	0.033	0.165	5.246	2.856	0.384	0.895
8	0.514	1.157	2.505	4.695	0.039	0.042
9	0.000	3.778	4.246	3.778	0.404	0.328
10	0.705	5.199	1.192	2.786	0.027	0.165

$$(12)\quad V = \sum_{j=1}^{n_L} \left[\frac{L_j(1 - L_j)}{N_t} \frac{q'_{ij}(1 - q'_{ij})}{n_j^*} + \frac{L_j^2 q'_{ij}(1 - q'_{ij})}{n_j^*} + \frac{L_j q'^2_{ij}}{N_t} \right] - \frac{p_i^2}{N_t}$$

where p_i = the proportion of age group i in the catch, L_j = the proportion of length group j in the catch, q'_{ij} = the proportion of fish of age i in length group j, N_t = the number of fish in the length sample, and n_j^* = the number of fish aged in length group j.

If all the sampled fish are age determined the variance of a proportion of an age group in a load can be estimated with

$$(13)\quad V = \frac{p_i(1 - p_i)}{n_s}$$

where n_s is the total number of age-determined fish.

If all the resources used in age and length determinations (255 h) had been used in age determinations, we would have been able to age 480 fish, an average of 48 fish per load. Because the estimates obtained with the age–length key were more accurate than those obtained with age readings only (Table 3), the variance estimates in this study may be conservative. Although the available dataset does not allow the exact estimation of the optimal proportion of age readings, it is obvious that the proportion of age readings was sufficient. Besides, by applying the age–length key, we obtained precise estimates of the length distributions of the catches.

Substantial gains in precision are possible with optimal allocation of sampling resources even if catches of a small-scale fishery are sampled. Our results show that age and length structures can be sampled contemporaneously without a noticeable loss in precision. Although optimal sampling scheme is dependent on the dynamics and structure of the population concerned, the principles outlined in this study may be useful if both age and length structures are desired.

Acknowledgements

We thank Pertti Eloranta and Mikael Hildén for their helpful comments concerning the manuscript and Seppo Kaitala for help with the graphs. We greatly appreciate the financial support for this study given by the City of Lahti.

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Changes in Maturity, Plasma Sex Steroid Levels, Hepatic Mixed-Function Oxygenase Activity, and the Presence of External Lesions in Lake Whitefish (*Coregonus clupeaformis*) Exposed to Bleached Kraft Mill Effluent

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Munkittrick, K. R., M. E. McMaster, C. B. Portt, G. J. Van Der Kraak, I. R. Smith, and D. G. Dixon. 1992. Changes in maturity, plasma sex steroid levels, hepatic mixed-function oxygenase activity, and the presence of external lesions in lake whitefish (*Coregonus clupeaformis*) exposed to bleached kraft mill effluent. Can. J. Fish. Aquat. Sci. 49: 1560–1569.

Lake whitefish (*Coregonus clupeaformis*) exposed to primary treated bleached kraft mill effluent (BKME) had reduced gonadal development and increased liver size relative to two reference populations. These results parallel our previous work on the white sucker (*Catostomus commersoni*) exposed to BKME at the same Lake Superior site. More detailed studies conducted in 1990, after the installation of an aeration stabilization basin at the pulp mill, found that lake whitefish exhibited reduced gonad sizes, delayed age to maturation, decreased levels of the plasma sex steroids testosterone and 17β-estradiol, and elevated hepatic mixed-function oxygenase (MFO) activity. Liver size was smaller following operation of the secondary treatment system. More than 20% of the lake whitefish collected at the BKME site in 1990 exhibited lateral, slash-like lesions which penetrated the body cavity. Histological examination revealed no evidence of an infectious etiology, and the wounds could not be accounted for by known causes. Similar lesions were found in 1991 near a second BKME discharge.

On a noté que des grands corégones (*Coregonus clupeaformis*) exposés à des effluents de pâte kraft blanchie relâchés d'usines de pâtes après épuration primaire montraient un moindre développement des gonades et une hypertrophie du foie par rapport à deux populations témoins. Ces résultats sont équivalents à ceux obtenus dans le cas du meunier noir (*Catastomus commersoni*) capturé au même endroit dans le lac Supérieur. Des études plus poussées menées en 1990 après l'installation d'un bassin d'aération ont révélé les aberrations suivantes chez le grand corégone : plus petites gonades, âge plus avancé au moment de la maturation, niveaux moindres des hormones stéroïdes sexuelles testostérone et oestradiol-17β dans le plasma et activité élevée de l'oxydase à fonction mixte dans le foie. Le foie était aussi de moindre taille après la mise en service du système d'épuration secondaire. Plus de 20 % des corégones capturés à cet endroit en 1990 portaient des entailles aux flancs pénétrant la cavité corporelle. Bien que l'examen histologique n'ait pas révélé de maladies infectieuses, la présence de ces entailles ne peut être expliquée. Des entailles semblables ont été relevées chez des poissons capturés en 1991 au voisinage d'un deuxième effluent de pâte kraft blanchie.

Received July 8, 1991
Accepted February 7, 1992
(JB117)

Reçu le 8 juillet 1991
Accepté le 7 février 1992

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There have been extensive descriptions of impacts of bleached kraft mill effluent (BKME) on fish populations in the Baltic Sea (reviewed in Södergren 1989), but until recently there was relatively little information available from field studies in North America. Studies conducted at Jackfish Bay, Lake Superior, in 1988 (Munkittrick et al. 1991) and 1989 (McMaster et al. 1991, 1992) revealed impacts of BKME on white sucker (*Catostomus commersoni*) at both the physiological and population level. White sucker exposed to BKME exhibited delayed sexual maturity, smaller gonads, reduced egg size, reduced secondary sexual characteristics, increased liver size, increased condition factor, and a smaller length at age than white sucker from comparable reference sites. White sucker exposed to BKME also exhibited reduced levels of plasma sex steroids (testosterone, 11-ketotestosterone, 17β-estradiol, and 17α,20β-dihydroxy-4-pregnen-3-one) and elevated levels of hepatic mixed-function oxygenase (MFO) activity as measured by the metabolism of benzo[*a*]pyrene, diphenyloxazole, and ethoxyresorufin.

A more recent study on the St. Maurice River found increased MFO activity and decreased testosterone levels in male white sucker collected downstream from a bleached kraft mill at La Tuque, Quebec (Hodson et al. 1992). Impacts of BKME on MFO activity have been reported in white sucker collected near other bleached kraft mills in Ontario (Smith et al. 1991; Servos et al. 1992). A fundamental question arising from these studies is whether similar responses to BKME are found in other teleost species. Elevated MFO activity has been reported for lake trout (*Salvelinus namaycush*; Munkittrick et al. 1992) and juvenile chinook salmon (*Oncorhynchus tshawytscha*; Rogers et al. 1989) exposed to BKME, but these studies did not include the measurement of circulating sex steroids and did not address impacts on reproduction.

Jackfish Bay receives the effluent from a 1200 tonne per day bleached kraft mill situated in Terrace Bay, Ontario. The pulp mill installed an aeration stabilization basin at a cost of more than $20 million that became operational in September 1989. Until the initiation of secondary treatment, the effluent entering Jackfish Bay from the mill had received only primary treatment (or no treatment) for 40 yr. This study had two main objectives: (1) to examine reproductive development in lake whitefish (*Coregonus clupeaformis*), a commercially important Lake Superior species, and (2) to compare the responses of fish collected during primary treatment (August 1989) with fish collected during the first year of secondary treatment (August 1990). Parameters measured were organ size, growth rates, MFO activity, and levels of plasma sex steroids.

Dioxins and furans and dioxin-like compounds have been shown to induce MFO activity and have been suggested as factors responsible for increasing MFO activity downstream of bleached kraft mills (Rogers et al. 1989; Hodson et al. 1992). As well as their MFO-inducing properties, mammalian studies with dioxins have shown them to induce immunotoxicity (Poland and Knutson 1982). Andersson et al. (1988) reported increased frequency of external pathological fin alterations and reduced white blood cell counts in perch (*Perca fluviatilis*) exposed to BKME, indicating a suppressed immune response. Because elevated dioxin levels (Sherman et al. 1990) induced MFO activity (Munkittrick et al. 1991; McMaster et al. 1991) and reduced concentrations of white blood cells (K. R. Munkittrick, unpubl. data) have been found in white sucker at this site, a survey of organs for histological evidence of impacts was conducted on lake whitefish.

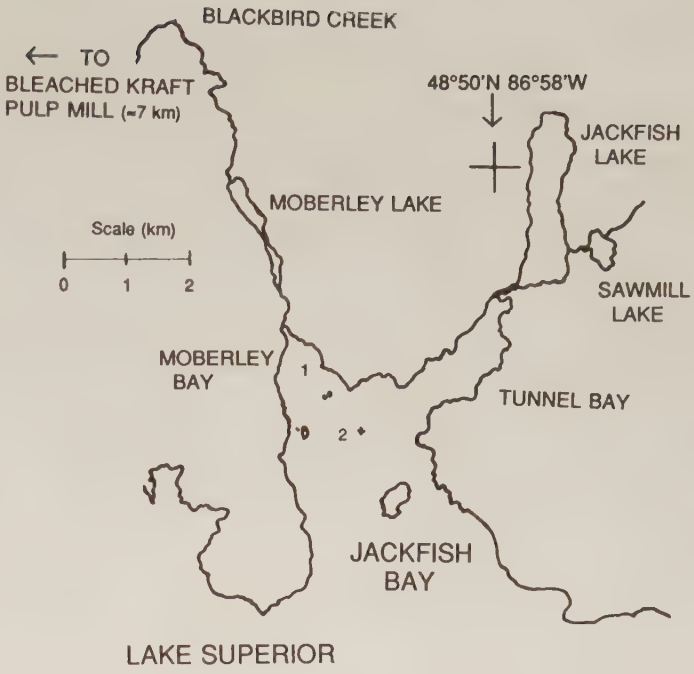

FIG. 1. Study site. The effluent from the bleached kraft mill enters Blackbird Creek and flows 15 km to Moberley Bay (1), the western arm of Jackfish Bay. Most of the lake whitefish were collected near Little Nick rock (2), approximately 1.8 km off the mouth of Blackbird Creek.

Methods

Effluent from the Terrace Bay mill reaches Jackfish Bay, Lake Superior, through Blackbird Creek (Fig. 1). Jackfish Bay has no residential or industrial development and receives no other industrial waste. Lake whitefish were collected in the summer from Jackfish Bay using gill nets (August 12–13, 1989; August 12–14 and September 27–30, 1990). Lateral, "slash-like" lesions were first noticed on a small number of fish collected in the effluent plume near the mouth of Blackbird Creek during August 1989. As lake whitefish are rare in these shallow waters, collections during August 1990 were made in deep water (>28 m) near Little Nick rock (approximately 1.8 km off the mouth of Blackbird Creek; Fig. 1). Reference collections were conducted in Mountain Bay (August 10–11, 1989; August 9–11, 1990; latitude 48°56′N, longitude 87°50′W) and Black Bay (August 20–21, 1989; October 1–3, 1990; 48°38′N, 88°32′W). Neither of these reference locations receives industrial effluent input or significant domestic effluent input, and both have been used previously to monitor BKME impacts (Smith et al. 1991). Complete reference study site maps are found in Munkittrick et al. (1991) and McMaster et al. (1991).

Calculation of exposure levels to BKME in Jackfish Bay is difficult, due to increased surface temperatures associated with the effluent, the presence of a strong thermocline in the summer months, and the influence of winds on mixing and distribution of the surface water plume. Based on data collected in 1987 and 1988 prior to secondary treatment (K. Sherman, Ontario Ministry of the Environment, Toronto, unpubl. data), surface dilutions were estimated to be 5:1 at the plume sampling site and 15:1 at Little Nick rock. However, these dilution calculations may be misleading. Chemical concentrations have only been determined for samples taken near the water surface, and the effluent plume is only present in the top 3–4 m of water at this time of year. Although exposure concentrations were not

determined in 1990, conductivity measurements taken in August 1991 declined from >2000 in the effluent to 1400 at the mouth of the creek and 80 at Little Nick; background levels were 75, suggesting a surface effluent concentration near Little Nick of 0.5%.

Both the white sucker and lake whitefish were captured at depths of 6 to >28 m of water and are benthic feeders as shown by identification of stomach contents (McMaster 1991 and M. E. McMaster, unpubl. data, respectively). Exposure concentrations for these species may be better indicated by sediment concentrations of contaminants. Total organic carbon (TOC) values in the effluent prior to secondary treatment ranged from 184 to 318 $\mu g \cdot mL^{-1}$ (Environment Ontario 1991), while sediment levels ranged from 400 $\mu g \cdot g^{-1}$ at the mouth of Blackbird Creek to 50–60 $\mu g \cdot g^{-1}$ at the plume station, 35–40 $\mu g \cdot g^{-1}$ at Little Nick, and 30 $\mu g \cdot g^{-1}$ at reference sites (K. Sherman, unpubl. data), corresponding to TOC levels of 5.4% at the plume station and 1.4% at Little Nick, relative to the mouth of Blackbird Creek.

During both years, fork length, total weight, liver weight, and gonad weight were determined for each fish. In 1990, the left operculum of each fish was removed and frozen. Opercula were later boiled and the annuli counted to determine their age. Blood was collected from live whitefish via caudal puncture into 5.0-mL heparinized vacuutainers and placed on ice for 6–8 h. The plasma was collected after centrifugation for 7 min, frozen in liquid nitrogen, and stored at −20°C prior to analysis. Levels of testosterone and 17β-estradiol in the plasma were measured by radioimmunoassay (RIA) following ether extraction. A description of the antisera used to measure testosterone is reported in Van Der Kraak et al. (1984) and for 17β-estradiol in Van Der Kraak et al. (1990). Measurement of 17β-estradiol and testosterone followed the description of Van Der Kraak et al. (1984). All plasma samples were assayed in duplicate, and interassay variability was less than 15% in both RIA systems.

Approximately 1 g of liver tissue was placed in cryovials, frozen immediately in liquid nitrogen, and stored at −80°C pending analysis. In the laboratory, the samples were thawed on ice and analyzed for hepatic cytochrome P450IA-dependent MFO activity using the catabolism of ethoxyresorufin (EROD) as described in McMaster et al. (1992).

External lesions were subjectively categorized on a scale of 1–7, with increasing numbers from 1 to 5 reflecting a progressive increase in the size and severity of the lesion and with numbers greater than 5 reflecting progressive healing. The numerical ratings are represented as follows: 1, represents a possible new lesion, not open; 2, a new lesion, not through to the peritoneal cavity; 3, a lesion into the peritoneal cavity, edges sharply defined; 4, a lesion into the peritoneal cavity, edges of wounds not sharp; 5, some healing has occurred to lesion; 6, new scar covers lesion; 7, old scar, well healed.

Analysis of covariance (ANCOVA) was used within years to test for differences between sites in condition factor (CF) (slope of fork length versus adjusted body weight [total weight − gonad weight − liver weight]). Differences in slopes of the regressions indicate differences in rates of body weight gain with length, indicating a difference in CF between sites. Differences in gonad weight and liver weight between sites were also tested using ANCOVA analyses, with adjusted body weight as the covariate. These data were first analyzed for homogeneity of slopes, and if slopes were similar (>0.05), the data were then tested for differences between intercepts, which signify differences between sites. In 1989, three sites were sampled (two reference, one contaminated). When intercept differences were present, direct contrasts between sites were completed to determine which sites were different. All analyses were completed using log-transformed values. Only five lake whitefish were captured at the reference site in September 1990, making analyses between sites difficult. Therefore, these analyses were completed only on August 1989–90 data. The September values are presented, however, to show their seasonal changes. Kruskal–Wallis analysis was used to determine differences between sites in age, hepatic EROD activities, and plasma testosterone and 17β-estradiol levels.

Results

Male lake whitefish were significantly younger at Jackfish Bay ($p = 0.007$) than at the Mountain Bay reference site in 1990; there was no difference in females ($p = 0.28$). There were no differences in CF between Jackfish Bay and the reference sites; slopes of regressions on fork length versus adjusted body weight were not different between sites for either males or females in 1989 ($p = 0.97$ and $p = 0.82$, respectively) or in 1990 ($p = 0.25$ and $p = 0.63$, respectively).

During 1989, both males and females were significantly shorter and lighter at the Mountain Bay reference site, and male and female fish from both reference sites had smaller livers and larger gonads than those from Jackfish Bay (Table 1). ANCOVA analysis of liver weight versus adjusted body weight in 1989 indicated no differences in the slopes for either males or females ($p = 0.91$ and $p = 0.34$, respectively). However, intercepts were significantly different between sites for both males and females ($p < 0.001$), indicating that liver weights were larger at the BKME site for both sexes, relative to both reference sites (Fig. 2). In 1990, this relationship was not evident, as intercepts were the same between sites for both males ($p = 0.08$) and females ($p = 0.13$), indicating similar liver sizes between sites (Fig. 2). This reduction in liver size is evident by the decline in the liversomatic index (LSI) from 1.94 to 1.35 in males and from 2.41 to 1.52 in females (Table 1) at the Jackfish Bay site from 1989 to 1990, while values from the reference site remained relatively constant.

Fish from the Mountain Bay and Black Bay reference sites had larger gonads relative to the BKME site in August 1989, as ANCOVA analysis indicated similar slopes ($p > 0.33$) but different intercepts for both males and females ($p < 0.001$) (Fig. 3). In 1990, similar trends were found, as intercepts were different ($p < 0.001$) between sites for both sexes; however, in male lake whitefish, the slopes of gonad weight versus adjusted body weight were different between sites ($p = 0.015$). In males, there was no difference in body weight between sites in August 1990; however, testicular size at the BKME site was only one third the size in fish from the reference site ($p < 0.001$; Table 1). The gonadosomatic indices (GSIs) of male and female lake whitefish collected at Jackfish Bay did not change following the initiation of secondary treatment (Table 1).

All the male lake whitefish collected from the reference sites were sexually mature; the smallest GSIs in these fish were approximately 1% (8 g in an 800 g fish; Fig. 3a). More than 90% of male whitefish (33/36 fish) collected at Jackfish Bay in August were immature, exhibiting low GSIs (<1% body weight), compared with 2/41 male fish at the reference sites which exhibited low GSIs (5% of fish) (Fig. 3a). This suggests a marked delay in sexual maturation at the Jackfish Bay site. Based on regressions of testes weight versus body weight, ref-

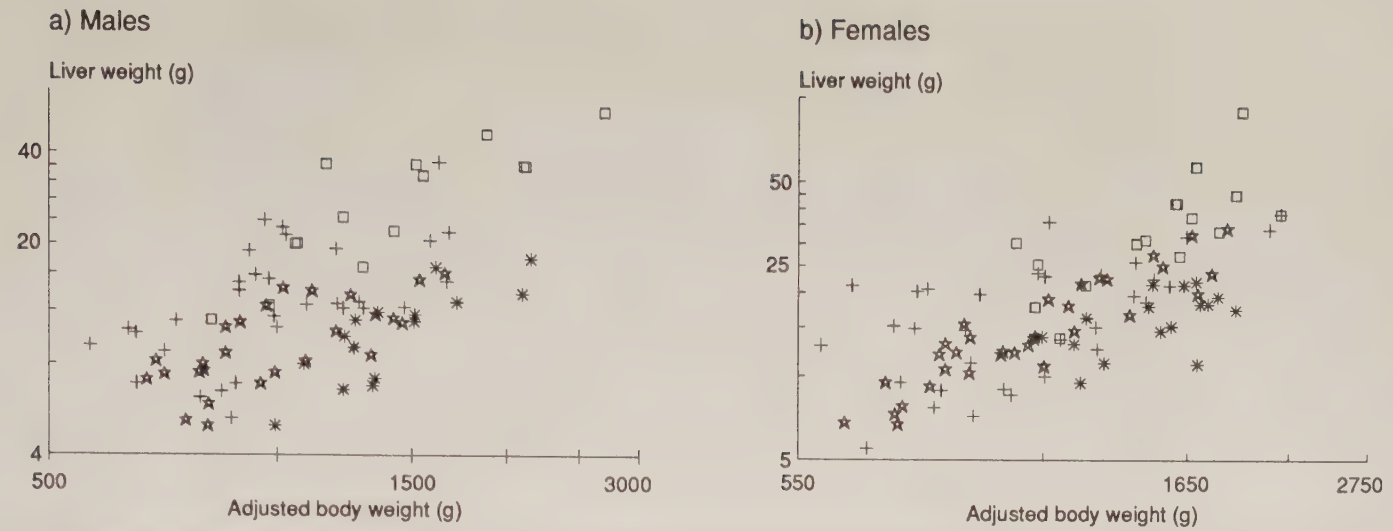

FIG. 2. Liver weight versus body weight for (a) male and (b) female lake whitefish collection from the BKME site (Jackfish Bay) in 1989 (boxes) or 1990 (crosses) relative to two reference sites (Mountain, stars; Black Bay, asterisks).

TABLE 1. Age, length, liver, gonad, and body weights for lake whitefish collected in August 1989 and August and September 1990 from the BKME-exposed (Jackfish) and reference (Black and Mountain) sites. Values are expressed as mean ± SE (*n*): asterisks denote a significant difference of the reference site from Jackfish Bay (BKME) for that year as analyzed by ANOVA using log-transformed values (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

Site	Date	Age (yr)	Length (cm)	Weight (g)	Liver (g)	LSI[a]	Gonad (g)	GSI[b]
Males								
Black	1989		46.3 ± 0.9 (15)	1445 ± 87 (15)	10.7 ± 1.0 (15)***	0.73 ± 0.04 (15)	28.6 ± 1.9 (15)***	2.02 ± 0.07 (15)
Mountain	1989		40.3 ± 1.4 (7)*	1039 ± 111 (7)*	11.0 ± 1.5 (7)***	1.07 ± 0.10 (7)	17.9 ± 2.6 (7)***	1.74 ± 0.13 (7)
	1990	8.95 ± 0.4 (14)**	42.7 ± 0.9 (18)	996 ± 63 (18)	9.0 ± 0.6 (18)*	0.93 ± 0.05 (18)	15.2 ± 1.9 (18)***	1.47 ± 0.11 (18)
Jackfish	1989		45.4 ± 1.5 (14)	1491 ± 143 (14)	29.2 ± 3.5 (14)	1.94 ± 0.14 (14)	7.3 ± 1.4 (14)	0.46 ± 0.06 (14)
	1990	7.6 ± 0.3 (32)	42.1 ± 0.7 (31)	999 ± 53 (31)	13.7 ± 1.4 (25)	1.35 ± 0.10 (25)	5.6 ± 1.4 (24)	0.46 ± 0.09 (24)
	September		43.7 ± 1.9 (9)	1211 ± 100 (10)	16.1 ± 1.7 (6)	1.53 ± 0.14 (6)	15.2 ± 4.3 (9)	1.19 ± 0.34 (9)
Females								
Black	1989		46.2 ± 0.7 (16)	1517 ± 60 (16)	16.3 ± 1.0 (15)***	1.16 ± 0.06 (15)	73.1 ± 8.0 (15)**	5.06 ± 0.49 (15)
Mountain	1989		42.1 ± 1.0 (16)**	1243 ± 67 (16)**	19.8 ± 1.8 (16)***	1.62 ± 0.07 (16)	51.6 ± 5.7 (16)**	4.24 ± 0.29 (16)
	1990	8.90 ± 0.5 (16)	41.6 ± 0.9 (17)	966 ± 82 (17)	15.5 ± 3.4 (17)	1.80 ± 0.49 (17)	47.3 ± 5.4 (16)**	5.00 ± 0.36 (16)
Jackfish	1989		45.9 ± 1.1 (16)	1520 ± 84 (16)	36.4 ± 4.5 (16)	2.41 ± 0.21 (16)	39.4 ± 5.7 (16)	2.58 ± 0.31 (16)
	1990	8.31 ± 0.4 (26)	42.9 ± 0.7 (27)	1125 ± 71 (27)	16.9 ± 1.9 (24)	1.52 ± 0.13 (24)	30.5 ± 5.8 (24)	2.45 ± 0.33 (24)
	September		42.1 ± 1.7 (10)	1124 ± 133 (10)	21.4 ± 1.9 (8)	2.22 ± 0.20 (8)	46.6 ± 23.3 (10)	3.78 ± 1.81 (10)

[a]LSI = liversomatic index = 100·liver weight/(body weight − liver weight).
[b]GSI = gonadosomatic index = 100·gonad weight/(body weight − gonad weight).

erence males would produce a 10-g gonad at a body weight of 742 g whereas a Jackfish Bay male would produce the same size gonad at 1615 g. Because Jackfish Bay lake whitefish grow at the same rate as reference fish, this represents a delay in maturity from 6.0 yr at the reference site to 10.4 yr, based on a regression of age versus weight for Jackfish Bay male lake whitefish (age = 0.005 weight + 2.28, $n = 24$, $r^2 = 0.77$). The maximum age of lake whitefish found was 12–13 yr.

Gonad weights in females showed similar site differences (Fig. 3b). All females from the reference sites had developing ovaries, with the smallest ovaries being approximately 20 g, representing a GSI of >2.5%. Almost 50% of the females from Jackfish Bay had GSIs below 2% and showed no evidence of ovarian development for the fall spawning period. As an indicator of delayed maturity, females at the reference sites would achieve a 20-g gonad during August at a weight of 549 g, compared with 994 g for females from Jackfish Bay (Fig. 3b). This corresponds to a delay in maturity from 5.5 yr at the reference site to 7.8 yr (age = 0.005 weight + 2.79, $n = 23$, $r^2 = 0.85$). However, mean fecundity estimates (August 1990 only) for Jackfish Bay (mean, SE, *n*: 26 728, 3010, 16) were higher than for Mountain Bay (14 294, 1750, 14) ($p = 0.002$). Although fecundity estimates were double at Jackfish Bay, mean gonadal weights were almost 50% lower (Table 1), suggesting that egg size was markedly reduced at Jackfish Bay.

Both BKME males and females had reduced plasma sex steroid levels in August 1990 (Fig. 4). Testosterone levels in males from Jackfish Bay were 3.4-fold lower than in those from the reference site ($p < 0.001$), while female testosterone and 17β-estradiol levels were 3.2-fold ($p < 0.001$) and 4.3-fold ($p < 0.001$) lower than the levels in females from the reference site. MFO activity (EROD) from Jackfish Bay was increased 11.6-fold in females ($p = 0.002$) and 5.2-fold in males ($p = 0.007$) relative to the reference site (Fig. 5).

Analyses for growth, gonadal, and liver differences between sites in September were not completed, as only five specimens were collected at the reference site in September, all of which were sexually immature (i.e. GSIs < 1%). This presumably reflects the proximity of collections to the spawning period and migration of mature adults to an unknown spawning area.

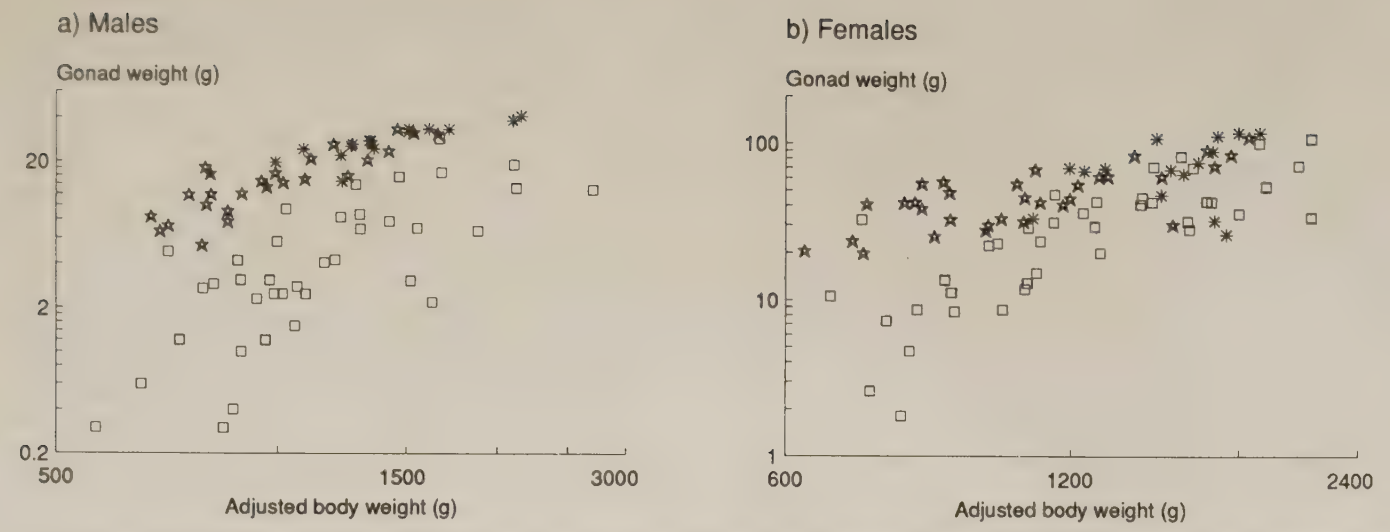

FIG. 3. Gonad weight versus body weight for (a) male and (b) female lake whitefish collected from the BKME site (boxes) and reference sites (Mountain, stars; Black Bay, asterisks). Data from 1989 and 1990 are pooled in this figure.

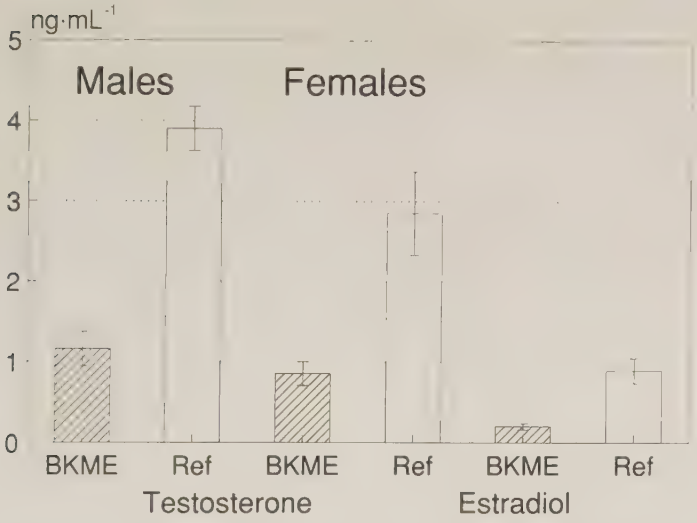

FIG. 4. Levels of plasma testosterone in male lake whitefish and testosterone and 17β-estradiol in females from Jackfish Bay (BKME; shaded) and Mountain Bay (reference; open) during August 1990.

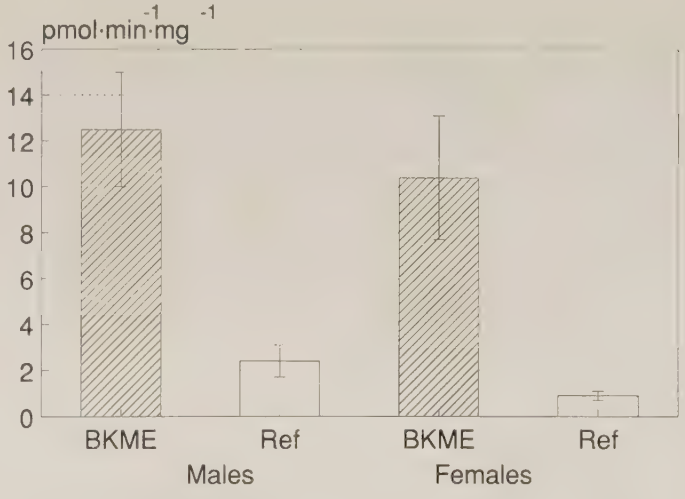

FIG. 5. Hepatic EROD activity (pmol resorufin·min^{-1}·mg protein^{-1}) of male and female lake whitefish collected from Jackfish Bay (shaded) and Mountain Bay (open) during August 1990.

Lesions

Lateral, elongated, slash-like lesions that penetrated the body cavity were evident on more than 20% (24/117) of lake whitefish collected during August 1990 (Fig. 6); no such lesions were found on fish from the reference sites. The 24 fish with lesions had a total of 31 lesions (three fish with two lesions, two fish with three lesions), of which 27 extended into the peritoneal cavity, or represented scars of wounds that had extended into the body cavity. Of the 18 lesioned fish sampled in detail during August 1990, there were no detectable effects of sex or size on lesion prevalence. Females constituted 56% of the samples and had average lengths and weights that were not significantly different from nonlesioned fish. The lesions were unilateral in 23 of 24 fish and were not symmetrical on the single fish with both sides lesioned. There was an equal prevalence on the right (15 lesions) and the left sides of fish (16 lesions). All lesions were on the body cavity, with 11 occurring in the pelvic region, 10 in the anterior region (below the lateral line), six in the posterior region, and four near the midline of the fish, extending to ventral areas (Fig. 7).

Histological sections of gill, liver, spleen, heart, and anterior and posterior kidney were examined from lake whitefish from Jackfish Bay (n = 11) and Mountain Bay (n = 14). Lesions in the gill, heart, spleen, anterior kidney, and liver were minor, consistent with those found in collection of wild fish, and no differences were detected between fish from the BKME and reference sites. The most striking changes were associated with the muscular lesions found on Jackfish Bay lake whitefish. Several of these lesions were examined microscopically, and all featured necrotic, waterlogged muscle, with mild lymphocytic inflammation of the dermis and an absence of epidermis (Fig. 8). No evidence of bacterial or fungal infection was visible in these sections. Coincident with this condition were marked changes in the posterior kidney, likely resulting from osmotic stress caused by the lesions. Several fish showed mild glomerular changes, with vacuolation and proteinaceous/hyaline casts deposited in the glomeruli (Fig. 9). The proximal tubule also contained proteinaceous material in some fish. More severe accumulations of hyaline material in the glomeruli were accompanied by glomerular swelling, dilation of the Bowman's space, and accumulations of hyaline material in the epithelium of both the proximal and distal tubules. Tubular and glomerular

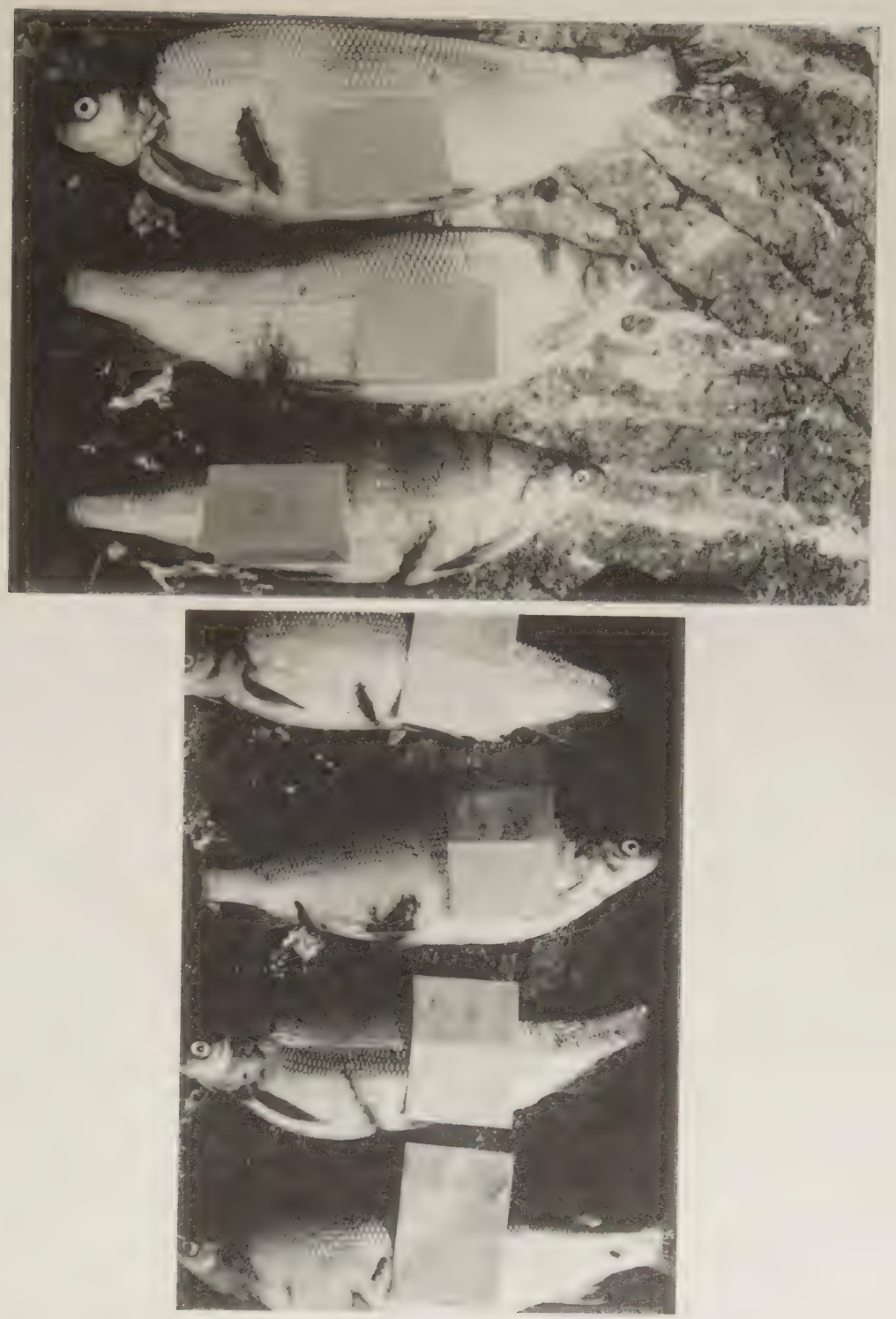

FIG. 6. Lesioned lake whitefish collected from Jackfish Bay in August 1990. The lesions were always on the body cavity and were present on more than 20% of fish examined.

necrosis was evident in only a single fish; distinct hyaline deposits were also evident in this fish, associated with the ellipsoids of the spleen and accumulations of melanomacrophages.

Discussion

The installation of an aerated lagoon (September 1989) and process changes since 1988 have reduced daily effluent discharges at the 1200 tonne per day mill from >35 000 kg of BOD, >5800 kg of total suspended solids (TSS), 59.4 kg of phosphorus (Ontario Ministry of the Environment 1988), and 2773 kg of AOX (T. Tseng, Environmental Protection, Environment Canada, Ottawa, Ont. K1A 0H3, pers. comm.) to 1500 kg of BOD, 4145 kg of TSS, 47.3 kg of phosphorus, and 1970 kg of AOX (Environment Ontario 1991). These reductions correspond to removal of 95% of BOD and 20–30% of the TSS, phosphorus, and AOX. This has resulted in elimination of acute lethality of the effluent to fish caged in Jackfish Bay (K. Flood, Ontario Ministry of Environment, Toronto,

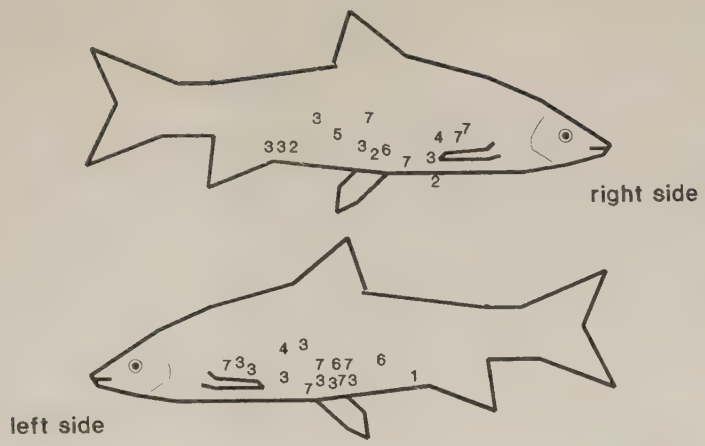

FIG. 7. Distribution and severity of lesions recorded in lake whitefish during August 1990. External lesions were subjectively categorized on a scale of 1–7, with increasing numbers from 1 to 5 reflecting a progressive increase in the size and severity of the lesion and with numbers greater than 5 reflecting progressive healing.

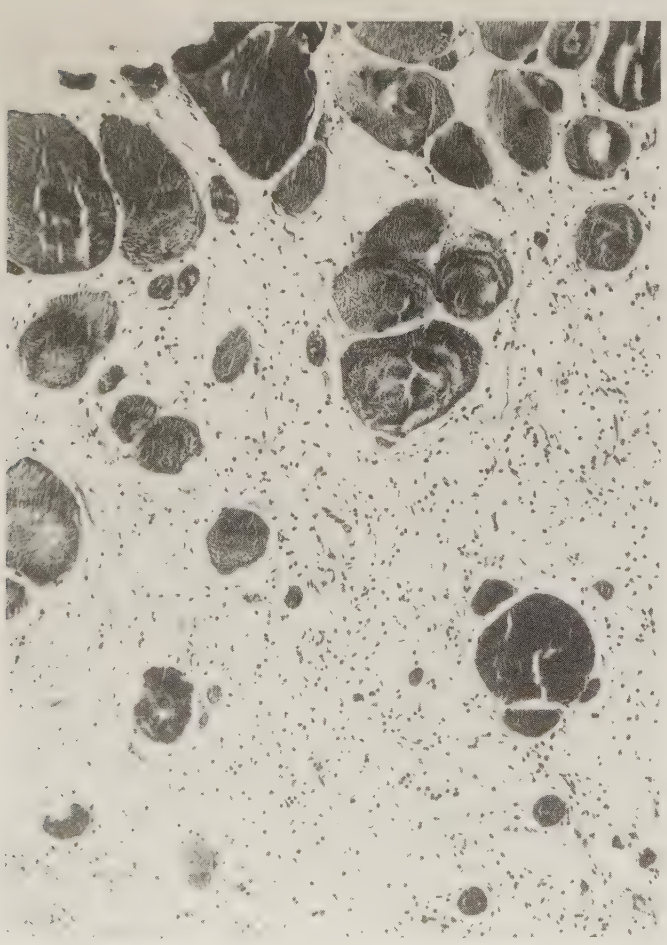

FIG. 8. Histological section through a lateral "lesion" on a lake whitefish showing intact muscle bundles among collagenous connective tissue. Note the absence of an inflammatory response. H&E, 1000× magnification.

unpubl. data) and has improved water clarity in Jackfish Bay and reduced the temperature of the discharge substantially. Despite the number of improvements, the biochemical parameters which we have monitored exhibit marked differences relative to those from reference locations.

Lake whitefish collected from Jackfish Bay showed significant increases in age at maturity, decreases in both gonadal size and plasma steroid levels, and increased levels of MFO activity. Similar changes have been reported in white sucker collected from this site during 1988–90 (Munkittrick et al. 1991; McMaster et al. 1991, 1992). Longnose sucker (*Catostomus catostomus*) also show depressed steroid levels in females, elevated MFOs in both sexes (Munkittrick et al. 1992), and an apparent delay in maturity (K. R. Munkittrick, unpubl. data). These results show that all three benthic species that have been examined in detail (lake whitefish, white sucker, longnose sucker) respond in a similar manner, although the magnitude of these changes differs between species.

Prior to the initiation of secondary treatment of BKME in September 1989, Jackfish Bay lake whitefish exhibited increased liver size relative to two reference sites. During the first year of secondary treatment (1990), liver weights decreased by 37% in females and >50% in males to values similar to those found at the reference sites, although hepatic MFO activity remained elevated. Similar changes were evident in white sucker, where liver size declined 47% in females and 22% in males in 1990 compared with 1989, although the livers were still larger than at the reference sites (K. R. Munkittrick, unpubl. data). This indicates that an increase in liver size is not required for induction of MFO activity. White sucker collected from 1988 to 1990 exhibited induced MFO activity, as measured by benzo[*a*]pyrene hydroxylase, diphenyloxazole hydroxylase, or EROD (Munkittrick et al. 1991, 1992; McMaster et al. 1991). There was no evidence of a decrease in MFO activity following installation of secondary treatment (Munkittrick et al. 1992). However, there was no evidence of MFO induction in longnose sucker, reduced MFO activity in white sucker, and a reduced impact zone for MFO induction in lake whitefish collected 2 wk after a planned mill maintenance shutdown (Munkittrick et al. 1992). These data suggest that (1) secondary treatment has not been successful in removing "MFO-active" compounds from BKME, (2) induction is not related to sediment contamination with persistent compounds, and (3) the inducing agent(s) are rapidly cleared by fish (Munkittrick et al. 1992).

Lake whitefish collected from Jackfish Bay in 1989 and 1990 had reduced gonad size relative to the two reference sites. This corresponds well with the reproductive impacts previously identified in Jackfish Bay white sucker (McMaster et al. 1991) and in perch at a site receiving BKME in the Baltic Sea (Andersson et al. 1988). Lake whitefish are fall spawners and normally spawn in late October/early November in Lake Superior. Fish captured in August are very well developed at this stage for the upcoming spawning season. The lack of any improvement in the reproductive commitment of lake whitefish from 1989 to 1990 in Jackfish Bay, the reduced levels of circulating sex steroids found in 1990, and the continued depression of gonad growth and steroid levels in white sucker during 1990 and 1991 (K. R. Munkittrick and G. J. Van Der Kraak, unpubl. data) suggest that 1–2 yr of secondary treatment has had no impact on the reproductive parameters that we have measured.

Examination of the ovaries from lake whitefish in August 1990 indicates that females from Jackfish Bay have significantly higher fecundity relative to the reference site. Although these fish have more eggs, their gonadal weights are significantly lower, indicating that eggs from Jackfish Bay are considerably smaller than those in reference females. White sucker from Jackfish Bay also have reduced egg size (McMaster et al.

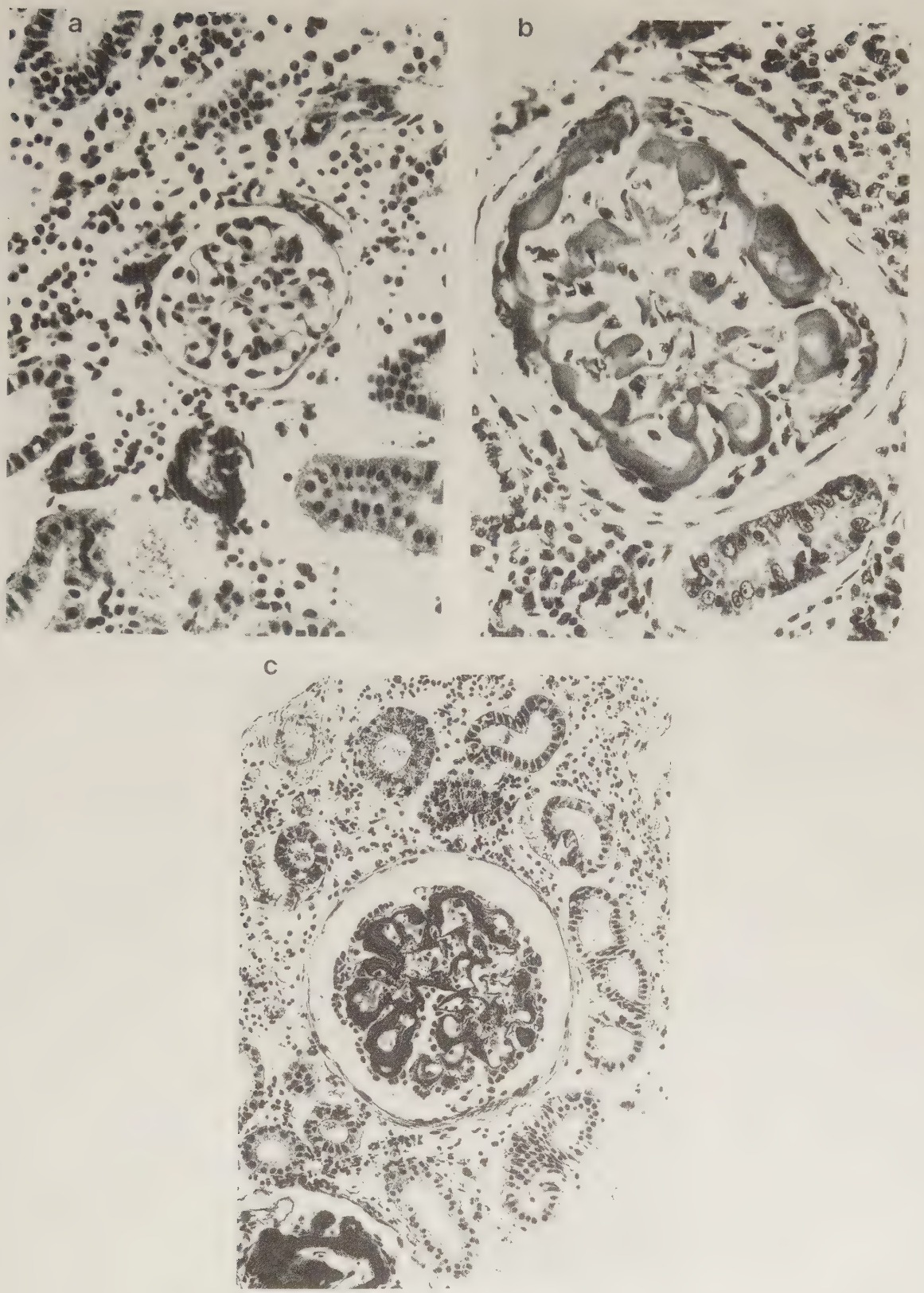

FIG. 9 (a) Normal renal glomeruli showing mild post-mortem (autolytic) vacuolation. Note the size of the glomerulus relative to adjacent renal tubules and the size of normal Bowman's space. H&E, 2000× magnification. (b and c) Renal glomeruli from lake whitefish with lateral "Lesion," showing distension of Bowman's space, glomerular swelling, and distension due to deposits of hyaline material adjacent to capillary endothelium. Note the enlargement of the glomeruli relative to adjacent tubule. H&E. Fig. 2b: 2000× magnification; Fig. 2c: 1000× magnification.

FIG. 10. Lake whitefish collected from the mouth of the Kam River, Thunder Bay, Lake Superior, showing the same type of lesions as seen in Jackfish Bay. The Kam River receives more than 2000 000 m^3 BKME·d^{-1}.

1992) but have reduced fecundity with age (Munkittrick et al. 1991). Although white sucker eggs were smaller at the BKME site, this had no effect on larval survival following hatch (McMaster et al. 1992). However, white sucker migrate to clean water to spawn, allowing the most sensitive stages of larval development to occur in uncontaminated waters. Lake whitefish, however, spawn in the lake and larval development may occur in contaminated areas. Whether there are impacts on early developmental life stages of lake whitefish is unknown.

More than 20% of the lake whitefish showed external lesions, in an equal frequency on both sides and sexes. The lesions were predominantly unilateral and never symmetrical, and most penetrated the body cavity without damage to underlying organs. The relative absence of inflammation suggests no infectious etiology for these lesions and/or a reduced ability to mount an immune response. The lesions are not seen on round whitefish (*Prosopium cylindraceum*), lake trout, lake herring (*Coregonus artedi*), white sucker, longnose sucker, or burbot (*Lota lota*), which are commonly captured in Jackfish Bay. Small numbers of northern pike (*Esox lucius*), chinook salmon, and walleye (*Stizostedion vitreum*) have also been collected at Jackfish Bay, but do not show external lesions.

Wounding of lake whitefish may occur during predatory attack by cormorants, loons, and other fish-eating birds. However, wounds associated with a successful escape from a predator have been oriented from anterior to posterior, not from dorsal to ventral (Reist et al. 1987). There is no known commercial fishing activity within Jackfish Bay and the wounds are too far posterior to be associated with gillnet marks. The presence of apparently fresh wounds in the population at the same time as healed scars suggests that some fish survive the wounding and that wounding is taking place at more than one time of year; wounds have been seen in Jackfish Bay during May, August, and September sampling periods and during 1989, 1990, and 1991 sampling years (K. R. Munkittrick, unpubl. data). The wounds are not related to spawning activity, since the lake whitefish were collected from relatively deep water (>35 m) in August, prior to spawning. There is no apparent explanation for the lesions.

During the fall and winter of 1990, photographs were distributed to numberous agencies, including the Ontario Ministry of Natural Resources (Lake Superior Fisheries Assessment Unit, Thunder Bay), Ontario Ministry of the Environment, Canadian Wildlife Service, and the Department of Fisheries and Oceans (Freshwater Institute, Winnipeg, Man.) as well as selected commercial fishermen operating on Lake Superior. The wounds were not recognized by either representatives of the various agencies or commercial fishermen who catch more than 10 000 lake whitefish per year from other areas of Lake Superior.

The presence of these lesions in an isolated location like Jackfish Bay, which has received large volumes of pulp mill effluent for more than 40 yr, suggests an association with the discharge of BKME. In May 1991, lake whitefish with similar lesions were collected from around the mouth of the Kaministikwia (Kam) River, Thunder Bay (Lake Superior), by personnel of the Lake Superior Fisheries Assessment Unit (P. Furlong, Ontario Ministry of Natural Resources, pers. comm.); the Kam River receives more than 200 000 m^3 of primary-treated BKME per day. Although commercial fishermen estimated the frequency of wounding at this site to be 20%, fish collections by the assessment unit found 18/224 (8%) of the lake whitefish to be lesioned. Seven specimens were sent to our laboratory, and the lesions appear similar to those seen in Jackfish Bay (Fig. 10). It is unknown if these lesions can be directly related to exposure to BKME. A survey of reference lake whitefish conducted during the 1991 spawning season at Mountain Bay found one fully healed wound out of 209 adults examined (one additional wound was an obvious fish predatory mark), suggesting that the wounding may be a natural phenomenon and that Jackfish Bay lake whitefish exhibit a decreased healing rate or increased wounding rate.

Summary

Lake whitefish exposed to BKME exhibited reduced gonad sizes, delayed age at maturation, decreased levels of the plasma sex steroids testosterone and 17β-estradiol, and induced hepatic

MFO activity. Liver size was smaller following operation of the secondary treatment system, but there was no other evidence of improvement during the first year of secondary treatment. The disappearance of MFO activity during a shutdown (Munkittrick et al. 1992) suggests that historical sediment contamination is not responsible for this observed physiological change. More than 20% of the lake whitefish collected at the BKME site exhibited lateral, slash-like lesions which penetrated the body cavity. Histological examination revealed no evidence of an infectious etiology, and no direct cause for the wounds could be found. Similar lesions were found in 1991 near a second BKME discharge on Lake Superior.

Acknowledgements

MFO measurements were conducted by Lynne Luxon (DFO, Burlington, Ont.). Funding for this study was provided by both the Department of Fisheries and Oceans and the Ontario Ministry of Environment Research Advisory Council. The assistance of Dave Rokosh (Water Resources Branch, OME, Rexdale, Ont.) was valuable. Field collections were supervised by Leo and Maye Marchand (Marchand Fisheries, Dorion, Ont.), and the assistance of Linda Melnyk-Ferguson (Ontario Ministry of Natural Resources, Terrace Bay, Ont.) and Lorne Townes (Ontario Ministry of Natural Resources, Nipigon, Ont.) are gratefully acknowledged. The samples and information provided by Pat Furlong, Lake Superior Fisheries Assessment Unit, Ontario Ministry of Natural Resources (Thunder Bay, Ont.), were appreciated. This report was prepared for the Ontario Ministry of Environment (MOE) and the Department of Fisheries and Oceans (DFO) as part of MOE- and DFO-funded projects related to Jackfish Bay. The views and ideas expressed in this report are those of the authors and do not necessarily reflect the views and policies of the provincial or federal government, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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Changes in Behaviour at Onset of Exogenous Feeding in Marine Fish Larvae

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Skiftesvik, A. B. 1992. Changes in behaviour at onset of exogenous feeding in marine fish larvae. Can. J. Fish. Aquat. Sci. 49: 1570–1572.

The activity and swimming speed of fed and starved larvae of Atlantic cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) were measured from hatching to metamorphosis. The results indicate changes in behaviour over time, as well as differences between starved and fed larvae. It is suggested that determining the point at which activity increases and swimming speed during active periods decreases may be relevant as an indicator of the time of first feeding in marine fish larvae.

On a quantifié le niveau d'activité et la vitesse de déplacement de larves nourries et de larves privées de nourriture de morue franche (*Gadus morhua*) et de turbot (*Scophthalmus maximus*), de l'éclosion des oeufs à la métamorphose. Les résultats révèlent des variations du comportement selon le temps écoulé, ainsi que des différences entre les larves nourries et celles privées de nourriture. On formule l'hypothèse que la détermination du moment où l'activité augmente et la vitesse de déplacement pendant les périodes d'activité diminue pourrait servir comme indicateur du moment de la première prise de nourriture chez les larves de poissons marins.

Received August 2, 1990
Accepted February 26, 1992
(JA671)

Reçu le 2 août 1990
Accepté le 26 février 1992

Atlantic cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) are two species in commercial culture where the timing of the introduction of prey is essential for subsequent growth and survival. Experiments have shown that for cod, first feeding is 4–5 d after hatching at 5°C (Ellertsen et al. 1980), while for turbot, this point is about 3 d after hatching at 18°C (Jones 1972). Successful predation on small zooplankton gives both new energy reserves and increased energetic outlay (Houde and Schekter 1980) which the larvae must to some extent control for further survival. Thus, one would expect not only ontogenetic changes in behaviour patterns but also changes in response to the feeding environment, particularly starvation. The following experiments were conducted to reveal changes in the behaviour of cod and turbot larvae from hatching to metamorphosis in response to feeding and starvation.

Materials and Methods

Sources of Larvae

Cod

Naturally spawned eggs from several adult cod from the broodstock held at the Austevoll Aquaculture Research Station were collected according to Huse and Jensen (1983) and incubated in a 250-L incubator at mean temperatures between 3 and 5°C. The eggs were transferred to observation chambers before hatching and observations began at 50% hatching (day 0). One group of 1500 larvae was starved and died out by day 15. The other group of 1500 fed larvae was followed to day 42. The remaining larvae were healthy enough to be maintained in aquaria until day 68 by which time cannibalism had made an impact on mortality. The fed group of larvae was given cultivated rotifers (*Brachionus plicatilis*) and *Tisbe* sp. nauplii on days 4–35 and natural zooplankton thereafter. The temperature was maintained at 5°C and salinity at 34 ppt. About 15% of the water was renewed daily. The light ws 300 lx at the surface.

Turbot

Turbot eggs were delivered from the Ardtoe Research Station, Scotland, and hatched in the observation chambers. Observations began 1 d after 50% hatching for the 200 larvae in the starvation group and ended on day 7, when the last larvae died. Observations on the 600 larvae in the fed group began 2 d after 50% hatching and continued until day 18. The fed larvae were given *B. plicatilis* and *Tisbe* sp. nauplii from day 3. The temperature was maintained at 18°C and salinity at 34 ppt. About 15% of the water was renewed daily. The light was 1000 lx at the surface.

Observations

The behaviour of cod and turbot larvae was defined as swimming or resting. Activity was defined as the percentage of time spent swimming during the total observation time. Swimming speed during active periods was defined as the average speed from commencement of activity to its cessation and was measured in millimetres per second. This was measured for 10 randomly chosen larvae during each observation period, as were the activity measurements.

Behavioural studies of marine fish larvae were conducted using a computer-aided video system, allowing three-dimensional measurement of the larvae's position in 70-L observation chambers (for a more detailed description, see Huse and Skiftesvik 1985, 1990). Each of 10 randomly chosen larvae was tracked for 5 min and position and behaviour (swimming or resting) logged for each second. This was repeated during daylight hours until termination of the experiments.

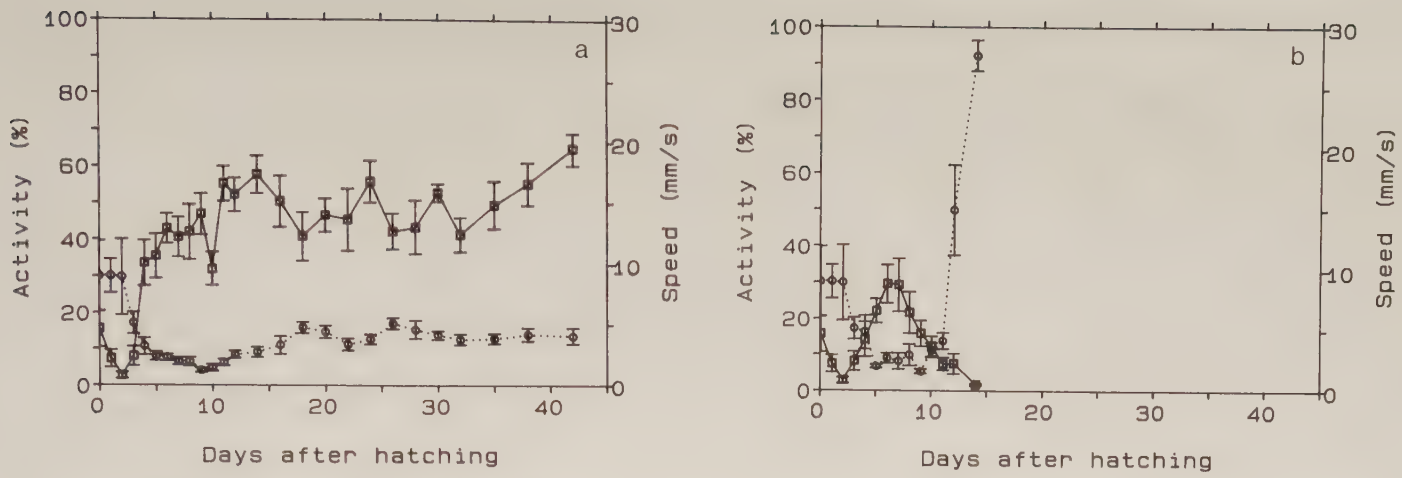

FIG. 1. Development of activity (solid line) and swimming speed (dotted line) in the active periods of (a) fed cod larvae and (b) starved cod larvae. Each point is the mean and standard error of 10 cod larvae.

Results

Cod

Activity

The activity of the two groups of cod larvae was the same throughout the first 3 d after hatching (under 10%) (Fig. 1). When prey were introduced to the fed group on day 4, the activity rose to over 30% and remained at that level for the remainder of the experiment (Fig. 1a).

The unfed group attained maximum activity of about 30% on days 6–7 but thereafter decreased within 4 d to under 10% (Fig. 1b). The last observation on d 14 shows an activity of about 2%.

Activity was significantly different between the two groups from day 4 onwards (robust *t*-test of medians; Hoaglin et al. 1983).

Swimming speed

The swimming speed during the active periods of both groups was identical during the first 4 d after hatching, being stable at 9 mm/s from day 0 to 3 and decreasing to 5 mm/s on day 4 (Fig. 1). In the fed group, the swimming speed during the active periods decreased further, remaining steady around 2 mm/s until about day 11 (Fig. 1a). Although the larvae developed in both length and morphology, the swimming speed during active periods did not exceed 5 mm/s for the rest of the experiment. The drop from day 3 to 5 was significant ($p < 0.0081$, Student's *t*-test).

The unfed group of cod larvae had comparatively elevated swimming speed during the entire observation period (Fig. 1b). On day 4, the mean speed during active periods was 5 mm/s. Speed dropped significantly ($p < 0.034$, robust *t*-test of medians; Hoaglin et al. 1983) on day 5 and remained at about the same level as in the fed group until day 10, when speed rose from about 4 to about 27 mm/s on day 14, the last day of observation.

Turbot

Activity

The activity was low (about 4%) for the first 3 d (Fig. 2) but rose on day 4, 1 d after the introduction of prey. No feeding was recorded on day 3. Thereafter, the turbot responded vigorously with activity higher than 70% from day 4 to 7 and over 90% for the remainder of the experiment (Fig. 2a). Activities of 100% were observed from day 16 to 18.

The unfed group had initially the same activity pattern as the fed group until the onset of exogenous feeding (Fig. 2b). Although activity rose on day 4, it was lower than in the fed group. Peak activity was on day 4 at about 55% and dropped to about 35% on day 7, the last day of observation.

Activity was significantly different between the two groups from day 5 onwards ($p = 0.0018$, Student's *t*-test).

Swimming speed

In contrast with the activity, the swimming speed in active periods was high during the initial observations (between 25 and 40 mm/s) (Fig. 2) but sank quickly to about 3.5 mm/s and increased slowly as larvae grow. The drop from day 3 to 4 was significant for both the fed and the unfed group ($p < 0.00005$, Student's *t*-test and $p < 0.00005$, robust *t*-test of medians (Hoaglin et al. 1983) for the fed and unfed group, respectively).

Discussion

The observations show a change in behaviour in both cod and turbot when they start feeding. Activity increases whereas swimming speed decreases. This change of swimming mode has also been found in larvae of other marine species when the energy source changed from endogenous to exogenous (Blaxter and Staines 1971; Hunter and Kimbrell 1980). Differences in activity were apparent at yolk absorption between fed and unfed larvae of black sea bream (*Acanthopagrus schlegeli*) (Fukuhara 1987). The swimming speed of these larvae also increased gradually as development proceeded and then slightly decreased after feeding began. Whereas fed larvae reduce their swimming speed, unfed larvae maintain less energetically conservative tempos when active, even exhibiting feeding behaviour until the day before mass mortality (Ellertsen et al. 1980).

The most obvious change in swimming speed between fed and unfed cod comes after day 10, a period near the point-of-no-return (days 8–10; Ellertsen et al. 1980). Turbot, according to Jones (1972), would have a point-of-no-return at day 7 at 18°C. However, a large change of swimming speed was not found for turbot. It is possible that some of the observed larvae in the fed group did not actually feed, although the narrow range of values for activity during the period would seem to

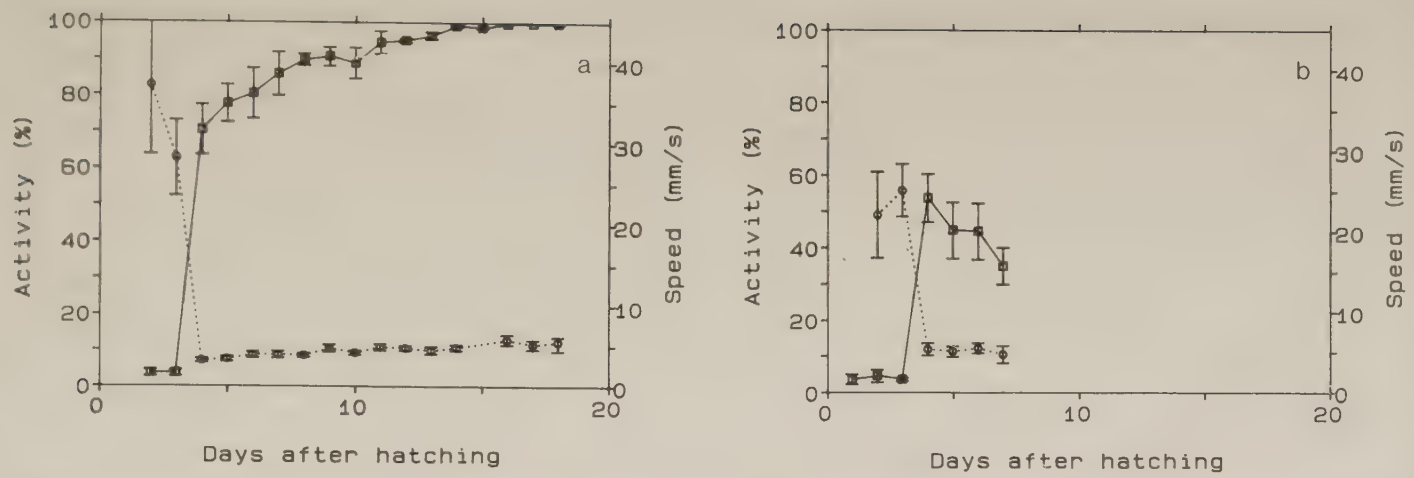

FIG. 2. Development of activity (solid line) and swimming speed (dotted line) in the active periods of (a) fed turbot larvae and (b) starved turbot larvae. Each point is the mean and standard error of 10 turbot larvae.

contradict this view. Thus it would seem that swimming speed alone is not sufficient as an early indicator of starvation.

Activity is correlated with the morphological development of the larva and may reflect the influence of predators. In both the fed and unfed groups of cod, activity rises between days 2 and 7, concurrent with the development of fins and the absorption of the yolk mass. If the activity of the unfed group is taken as baseline, then the presence of prey augments the activity levels by about 15% during days 4–7, during the changeover from endogenous to exogenous food. When the yolk supply has been exhausted, the unfed larvae conserve their energy by reducing activity after day 7 whereas the fed larvae maximize their contact probability by increasing their activity.

For both cod and turbot larvae, the activity was lower in the unfed group compared with the fed group after first feeding. Reduced swimming activity of unfed cod larvae was also found by Ellertsen et al. (1980). In studies cited by Sih (1987), reduced activity was a typical antipredator response that presumably reduces the frequency of contact with predators and the likelihood of detection and recognition as prey. Low activity also implies reduced consumption of energy; thus, reduced activity may delay the point-of-no-return.

From studies by Milinski (1986) and Jakobsen et al. (1988), it is known that fish that are starved, or by other reasons have an increased need for exogenous energy, will take higher risks. The buoyancy of starved larvae is different from that of fed larvae (Blaxter and Erlich 1974). Thus, starved larvae must have a vertical distribution different from that of fed larvae, as shown by Neilson et al. (1986). This may indicate that the demand for exogenous energy becomes more important than predator avoidance.

In summary, there is a change in the activity and speed of cod and turbot around the time of first feeding. This may reflect the increasing importance of energy acquisition as a motivator, in addition to ontogenetic changes. It is suggested that determination of the period during which activity increases and swimming speed decreases may be used to determine the time of first feeding in marine fish larvae.

Acknowledgements

The author would like to thank Karin Pittman (Department of Fisheries Biology, University of Bergen), Øivind Bergh, Jens Chr. Holm, and Ingvar Huse (Austevoll Aquaculture Research Station, Storebø) for constructive contributions to the manuscript. The Norwegian Council for Fisheries Research (NFFR) supported the author financially.

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Effectiveness of a Stock Enhancement Program Evaluated by a Two-Stage Sampling Survey of Commercial Landings

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In this study, we evaluated the effectiveness of fish stock enhancement programs using a two-stage random sampling survey of commerical landings. Estimators for the total number of fish landed, the ratio of the number of fish released to the total number of fish landed, the recovery rate, and the amount of income were formulated. Variance formulas for these estimators were also derived. In a case study of the flounder fishery, we obtained the conservative estimate of 36 982 recoveries from 246 300 hatchery-reared fish over 3 yr after release. The ratio of the recovery rate to the number of fish released was 0.15. The total income and benefit estimate were $260 000 and $63 000 (U.S.), respectively. We found that the stock enhancement program was economically profitable. We also studied several sampling strategies. We recommend that as many markets as possible be surveyed for a minimum number of days because the variation in daily landings among markets was larger than that within markets. Stratification of markets should be conducted for accurate estimation.

Dans la présente étude, nous avons évalué l'efficacité des programmes d'amélioration des stocks de poissons à l'aide d'une enquête par échantillonnage aléatoire en deux étapes sur les débarquements commerciaux. Nous avons élaboré des formules permettant d'estimer le nombre total de poissons débarqués, le rapport du nombre de poissons relâchés au nombre total de poissons débarqués, le taux de récupération et le revenu. Nous avons également élaboré des formules pour exprimer la variance de ces paramètres. Dans une étude du cas des pêcheries de la plie, nous avons obtenu une estimation raisonnable de 36 982 poissons récupérés, à partir de 246 300 poissons élevés en écloserie, trois ans après leur libération. Le rapport du taux de récupération au nombre de poissons relâchés était égal à 0,15. Les revenus et les bénéfices totaux étaient respectivement estimés à 260 000 $ (É.-U.) et 63 000 $ (É.-U.). D'après nos résultats, le programme d'amélioration des stocks est économiquement rentable. Nous avons également étudié plusieurs stratégies d'échantillonnage. Nous recommandons d'étudier le plus grand nombre possible de marchés pendant un nombre de jours minimum, car la variation des débarquements quotidiens d'un marché à l'autre est plus élevée que celle à l'intérieur des marchés. Pour obtenir une estimation précise, il faudrait procéder à une stratification des marchés.

Received July 29, 1991
Acepted February 26, 1992
(JB165)

Reçu le 29 juillet 1991
Accepté le 26 février 1992

The current Japanese stock enhancement programs for coastal marine resources were initiated in 1963 in response to a decreasing trend in landings of important fish and shellfish species. In this program (termed "Saibai-Gyogyo" in Japanese), hatchery releases of fingerlings have been conducted. Stock enhancement for species in Japanese coastal waters and aquaculture both rose in importance with the declaration of the 200-mile Exclusive Economic Zone ratified by many countries. The annual production from Japanese aquaculture reached 1.27 million tons in1989 and accounts for 11% of all Japanese annual landings for marine fisheries. However, few suitable sites for aquaculture remain around the Japan coast. The Japanese government has therefore given a good deal of encouragement to the stock enhancement program. In Norway, enhancement studies of coastal cod have been carried out since 1983 (Kristiansen and Svåsand 1990; Svåsand et al. 1990; Svåsand and Kristiansen 1990a, 1990b). In the United States, Polovina (1990) studied the contribution of released juveniles to a rockfish fishery by simulation models. However, there are no studies that directly estimate the recovery rate and the benefit of an enhancement program by a survey of landings in fish markets.

At present, the Saibai-Gyogyo program covers 92 species (salmonids are not included). The present status of the project was reviewed by Matsuoka (1989a) and Suda (1990). The enhancement programs are conducted mainly at 14 hatchery facilities of the Central Government and also at 50 hatcheries of 39 coastal prefectures. In addition, 16 city, town, and village governments and 94 fishermen's cooperative associations have their own facilities. Techniques to produce fingerlings have been developed for many species. Matsuoka (1989b) described the current state of the fingerling production in Japan. In 1989, reared fingerlings of 80 species were released in coastal Japan (Fishery Agency and Japan Sea-Farming Association 1991).

TABLE 1. Number of fingerlings released for major species (unit: 1000 individuals).

Species	1983	1984	1985	1986	1987	1988	1989
Red seabream	15 624	16 176	12 655	17 248	23 712	17 376	16 080
Flounder	3 278	4 521	4 621	6 626	8 822	8 865	10 893
Prawn	300 585	293 620	291 909	306 882	336 933	323 964	288 747
Swimming crab	19 523	20 032	27 101	30 067	20 639	26 296	29 722
Scallop	1 607 213	1 776 130	2 009 893	2 192 170	2 926 131	2 027 969	3 231 386
Abalone	18 334	19 018	19 431	21 278	22 398	20 579	22 659

Because the number of fingerlings of several species is continually increasing (Table 1), an evaluation of the effectiveness of the stocking program is necessary.

Marine stock enhancement programs have a long history. Hatchery releases of yolk-sac larvae of cod were started in Norway and the United States at the end of the last century (Solemdal et al. 1984). In Japan, releases of hatched-out larvae of cod, herring, and king crab were conducted in the early 1900's (Ohshima 1983). However, several studies have failed to detect a positive result from these releases. The effectiveness of the stock enhancement programs has been evaluated by several methods. A review of the procedures for the evaluation was carried out by Matsumiya (1986). The methods of evaluation were classified into four groups (Kitada 1991). One of the groups, the sampling survey of commercial landings, is treated in this article. The other three groups are as follows.

(1) Estimation of total recoveries based on tag recoveries reported by fishermen (Takama 1986). Tag recoveries are corrected for the rate of reporting and shedding of tags.

(2) Methods based on a correlation between the annual number of fingerlings released and the annual landing weights (Ishibashi 1984; Kitada 1984; Takama 1986).

(3) Prediction of total recoveries by calculating yield per release based on catch equation models (Hiyama 1975; Kitada 1983). In recent years, release strategies have been evaluated by simulation models (Watanabe 1988; Matsumiya and Ohnishi 1989; Kitada and Ohkouchi 1990; Polovina 1990).

We believe that proper estimates of recovery cannot be obtained by the above methods for several reasons. Regarding the first method, tag recoveries have often been used in survival estimation studies (Kitahara et al. 1986; Kitada and Hirano 1987; Svåsand and Kristiansen 1990b). Although this type of estimation is useful in studies of the rate of recruitment to a wild population, especially during the early period after release, it is not effective for estimating the total recovery of released fish because the reporting rate is very low and the rate of shedding of tags is large (Kitada and Suda 1988). In addition, these rates vary with the time elapsed and it is difficult to estimate them properly. The evaluation by Method 2 is performed on the assumption that the annual catch depends on the number of individuals released. When the recruitment rate of wild fish is relatively stable among years and the annual number of released fish is large enough compared with the recruitment in each year, the assumption is valid. However, this is not true in most cases. Furthermore, the assumption is also invalid because the annual catch consists of several year-classes. Simulation models predicting effectiveness (Method 3) are valid for evaluating stocking strategies, but the actual recovery from released fish is not estimated.

To evaluate the impact of stocking, it is necessary to estimate the relationship between appropriate measures of the effectiveness and stocking variables such as the number or size or time or site of stocking. Three measures are considered. The survival rate of fish stocked and the mark ratio (ratio of marked fish stocked to the total landings) are the relative measures of effectiveness. For direct evaluation, the recovery rate (the ratio of stocked fish recovered to the total number of fish stocked) is important, and a proper estimate of the number of stocked fish recovered is needed. It is difficult to estimate these measures for crustaceans because the released fingerlings cannot be identified with tags. In recent years, efforts have been made to survey and count the number of released fish recovered by age in commercial landings at fish markets (Shiihara 1986). This direct approach of estimating the number of fish recovered is considered the most reliable. However, the survey procedure and the method of estimation hae not been fully investigated. Kitada (1986) considered estimating of the ratio of the number of marked fish to the total landings, but variation among landing days and among markets was not fully taken into account. In this article, we propose a direct method for the estimation of stocking effectiveness by a two-stage sampling survey of markets and survey days. The estimator and variance for the mark ratio, recovery rate, and the benefit from released fish were derived. Deriving the variance formula as a function of the number of markets surveyed and the survey days, we see that we should survey as many markets as possible and minimize the number of survey days in each market. As an example, we applied this procedure to the case of the flounder (*Paralichthys olivaceus*) in Fukushima Prefecture. Several sampling strategies are discussed.

Current Survey Procedures and Two-Stage Sampling Scheme

In general, released fish migrate widely and are caught by various kinds of fishing gear and landed at many fish markets of fishermen's cooperative associations. Landings are sorted by species and sold to buyers on every landing day. The total landing weight for each species is recorded in the fish market, but the total landings and the age composition of the catch are unknown. Usually, samplers go to the markets at prearranged times to survey the landings. Because it is difficult to survey all markets on all landing days, these are selected according to certain criteria. Commonly the markets that have large landings and those using the fishing gear targeting the species under study are selected. Geographical accessibility is also taken into account. Thus, market selection is not random but purposive. Furthermore, surveys are conducted on only a few days out of every month. On a selected survey day, all individuals of the target species in the landings are classified as to whether they are wild or artificially produced by their marks, and their ages are determined from body length data.

In this way, we can classify the fish in all landings at any market. In general, it is possible to survey all fish of a given hatchery-released species during a survey day because hatchery-released species are expensive and the daily landigs are usually not large. However, the markets are geographically dispersed. Taking into account the cost of and time for sampling,

a simple random sampling of landings is difficult. It is convenient to select markets and survey the landings of these markets on days selected beforehand. This is two-stage sampling with markets as a primary sampling unit and landing days as a secondary sampling unit. However, the precision of estimates obtained by two-stage random sampling may be lower than that of a simple random sampling when there are large variances among markets. To overcome this problem, stratification of markets is carried out.

Picquelle (1985) considered the estimators for spawning parameters of adult northern anchovy by two-stage sampling, where the primary sampling units were the operating location of a trawler and the secondary units were the number of adult fish in the trawler net. Sen (1986) also proposed a two-stage sampling plan for estimating the total number of landings of rockfish in each port. In this case, the sampling units were boat trips and fish boxes.

In this study, we estimate the total landings for both released and total fish and the mark ratio. By conducting successive surveys and estimating the number of landings of released fish by age, we can evaluate the economical effectiveness of quantities such as the recovery rate. It is important to determine, considering costs and benefits, the number of markets and days that must be sampled to achieve sufficient precision.

Estimation and Evaluation of Precision

Total Landings and Ratio of Marked Fish in those Landings

Consider estimation of the total number of released fish in the landings and the mark ratio for a given species in a given survey period. We treat a case in which all fingerlings are released with marks because the natural population is larger than the number of fish released in almost all stocking plans. Let us define notations as follows, omitting every suffix for age:

K = total number of markets in the survey area
k = number of markets selected for the survey of landings
D_i = total number of landing days in the ith market
d_i = number of days sampled at the ith market
M = total number of released fish in the landing (only released fish)(to be estimated)
N = total number of fish in the landing (released fish plus wild fish)(to be estimated)
R = ratio of marked fish in the landings (mark ratio) ($= M/N$) (to be estimated)
M_i = total number of released fish in the landing in the ith market
N_i = total number of fish in the landing in the ith market
m_{ih} = number of released fish in the landing in the hth day in the ith market
n_{ih} = total number of fish in the landing in the hth day in the ith market.

We denote the jth market in the sample by $i(j)$ and the sth day sampled as $h(s)$. The suffix of h depends on the market selected but we do not use $h_{i(j)}$ for simplicity.

M, N, and R are defined as

$$M = \sum_{i=1}^{K} \sum_{h=1}^{D_i} m_{ih}$$

$$N = \sum_{i=1}^{K} \sum_{h=1}^{D_i} n_{ih}$$

$$R = \frac{M}{N}.$$

When the markets and landing days to be surveyed are sampled with equal probabilities, respectively, unbiased estimators of M and N are obtained by

$$(1) \quad \hat{M} = \frac{K}{k} \sum_{j=1}^{k} \frac{D_{i(j)}}{d_{i(j)}} \sum_{s=1}^{d_{i(j)}} m_{i(j)h(s)}$$

$$(2) \quad \hat{N} = \frac{K}{k} \sum_{j=1}^{k} \frac{D_{i(j)}}{d_{i(j)}} \sum_{s=1}^{d_{i(j)}} n_{i(j)h(s)}.$$

From these, we get the ratio estimator of R as

$$(3) \quad \hat{R} = \frac{\hat{M}}{\hat{N}}.$$

The bias of $\hat{R}$ is neglected when the sample size is large.

Supposing simple random sampling without replacement in each stage, the variance of $\hat{M}$ is given by

$$V(\hat{M}) = \frac{K}{k} \sum_{i=1}^{K} D_i^2 \frac{D_i - d_i}{D_i - 1} \frac{\sigma_{M_i}^2}{d_i} + K^2 \frac{K - k}{K - 1} \frac{\sigma_{M_b}^2}{k}$$

where

$$\sigma_{M_i}^2 = \frac{1}{D_i} \sum_{h=1}^{D_i} (m_{ih} - \mu_i)^2$$

$$\sigma_{M_b}^2 = \frac{1}{K} \sum_{i=1}^{K} (M_i - \bar{M})^2$$

and $\mu_i = \frac{1}{D_i} \sum_{h=1}^{D_i} m_{ih}$, $\bar{M} = M/K$. This expression is equivalent to Cochran's expression (11.22)(Cochran 1977, p. 303).

The variance estimate for $\hat{M}$ is derived as

$$(4) \quad \hat{V}(\hat{M}) = \left(\frac{K}{k}\right)^2 \sum_{j=1}^{k} D_{i(j)}^2 \frac{D_{i(j)} - d_{i(j)}}{D_{i(j)} - 1} \frac{\hat{\sigma}_{M_{i(j)}}^2}{d_{i(j)}} + K^2 \frac{K - k}{K - 1} \frac{\hat{\sigma}_{M_b}^2}{k}$$

where $\hat{\sigma}_{M_{i(j)}}^2$ is the unbiased estimator of $\sigma_{M_i}^2$, that is

$$\hat{\sigma}_{M_{i(j)}}^2 = \frac{1}{d_{i(j)} - 1} \sum_{s=1}^{d_{i(j)}} (m_{i(j)h(s)} - \hat{\mu}_{i(j)})^2$$

$$\hat{\mu}_{i(j)} = \frac{1}{d_{i(j)}} \sum_{s=1}^{d_{i(j)}} m_{i(j)h(s)}.$$

The between-variance among markets for landings of released fish is evaluated unbiasedly as

$$(5) \quad \hat{\sigma}_{M_b}^2 = \frac{1}{k} \sum_{j=1}^{k} (\hat{M}_{i(j)} - \tilde{M})^2 - \frac{k - 1}{k^2} \sum_{j=1}^{k} D_{i(j)}^2 \frac{D_{i(j)} - d_{i(j)}}{D_{i(j)} - 1} \frac{\hat{\sigma}_{M_{i(j)}}^2}{d_{i(j)}}$$

where $\hat{M}_{i(j)} = D_{i(j)} \hat{\mu}_{i(j)}$ and $\tilde{M} = \frac{1}{k} \sum_{j=1}^{k} \hat{M}_{i(j)}$ (see Appendix). We separately evaluated the unbiased estimators of

within- and between-variances to discuss the sample sizes of primary and secondary units. The estimator

$$\tilde{\sigma}^2_{M_b} = \frac{1}{k} \sum_{j=1}^{k} (\hat{M}_{i(j)} - \tilde{M})^2$$

is biased. Cochran (1977) derived the unbiased estimator of the between-variance in the simplest case in which every unit contains the same number of subunits (see Cochran 1977, p. 279).

In a similar way the variance for $\hat{N}$ is obtained by

$$(6) \quad \hat{V}(\hat{N}) = \left(\frac{K}{k}\right)^2 \sum_{j=1}^{k} D^2_{i(j)} \frac{D_{i(j)} - d_{i(j)}}{D_{i(j)} - 1} \frac{\hat{\sigma}^2_{N_{i(j)}}}{d_{i(j)}} + K^2 \frac{K - k}{K - 1} \frac{\hat{\sigma}^2_{N_b}}{k}$$

where

$$\hat{\sigma}^2_{N_{i(j)}} = \frac{1}{d_{i(j)} - 1} \sum_{s=1}^{d_{i(j)}} (n_{i(j)h(s)} - \hat{\nu}_{i(j)})^2$$

$$\hat{\nu}_{i(j)} = \frac{1}{d_{i(j)}} \sum_{s=1}^{d_{i(j)}} n_{i(j)h(s)}$$

and the variance among markets for total landings is

$$(7) \quad \hat{\sigma}^2_{N_b} = \frac{1}{k} \sum_{j=1}^{k} (\hat{N}_{i(j)} - \tilde{N})^2 - \frac{k - 1}{k^2} \sum_{j=1}^{k} D^2_{i(j)} \frac{D_{i(j)} - d_{i(j)}}{D_{i(j)} - 1} \frac{\hat{\sigma}^2_{N_{i(j)}}}{d_{i(j)}}.$$

The covariance between total and released fish in the landing is also estimated by

$$(8) \quad \widehat{\mathrm{Cov}}(\hat{M}, \hat{N}) = \left(\frac{K}{k}\right)^2 \sum_{j=1}^{k} D^2_{i(j)} \frac{D_{i(j)} - d_{i(j)}}{D_{i(j)} - 1} \frac{\hat{\sigma}_{MN_{i(j)}}}{d_{i(j)}} + K^2 \frac{K - k}{K - 1} \frac{\hat{\sigma}_{MN_b}}{k}$$

where

$$\hat{\sigma}_{MN_{i(j)}} = \frac{1}{d_{i(j)} - 1} \sum_{s=1}^{d_{i(j)}} (m_{i(j)h(s)} - \hat{\mu}_{i(j)})(n_{i(j)h(s)} - \hat{\nu}_{i(j)})$$

and $\hat{\sigma}_{MN_b}$ is obtained by

$$(9) \quad \hat{\sigma}_{MN_b} = \frac{1}{k} \sum_{j=1}^{k} (\hat{M}_{i(j)} - \tilde{M})(\hat{N}_{i(j)} - \tilde{N}) - \frac{k - 1}{k^2} \sum_{j=1}^{k} D^2_{i(j)} \frac{D_{i(j)} - d_{i(j)}}{D_{i(j)} - 1} \frac{\hat{\sigma}_{MN_{i(j)}}}{d_{i(j)}}.$$

The asymptotic variance estimate of $\hat{R}$ is given by

$$(10) \quad \widehat{\mathrm{MSE}}(\hat{R}) \simeq \hat{R}^2 \left\{ \frac{\hat{V}(\hat{M})}{\hat{M}^2} + \frac{\hat{V}(\hat{N})}{\hat{N}^2} - \frac{2\widehat{\mathrm{Cov}}(\hat{M}, \hat{N})}{\hat{M}\hat{N}} \right\}.$$

To obtain smaller variances for these estimates, a stratified simple random sampling procedure is efficient. The estimates of the total population and their variances and covariances are obtained by simple addition of the estimates of the strata. The ratio estimate and its variance are obtained from these estimates across strata. If we suppose that variations among markets and seasonal changes in the amount of landings exist, then stratifications for these estimations should be valid.

Recovery Rate

By conducting successive surveys over several years, the total number of recoveries of released fish can be estimated yearly. We can estimate the survival rate of released fish by examining the time series data of the estimate for each cohort.

In particular, it is important to evaluate the efficiency from the economic point of view. For example, the total cash value of recoveries for a given cohort (age 0 to A) is estimated by

$$\hat{S} = \sum_{a=0}^{A} \hat{M}_a \hat{W}_a C_a$$

where M_a is the number of released fish in the landing of age a released at age 0, W_a is the mean body weight of individual fish of age a, and C_a is a mean price per unit weight. We recommend estimating M_a by month because W_a and C_a fluctuate largely among seasons.

The variance estimate of $\hat{S}$ is

$$(11) \quad \hat{V}(\hat{S}) = \sum_{a=0}^{A} (\hat{W}_a C_a)^2 \hat{V}(\hat{M}_a) + \sum_{a=0}^{A} (\hat{M}_a C_a)^2 \hat{V}(\hat{W}_a) + 2 \sum_{a<a'}^{A} \hat{W}_a \hat{W}_{a'} C_a C_{a'} \widehat{\mathrm{Cov}}(\hat{M}_a, \hat{M}_{a'}).$$

Assuming that M_a and $M_{a'}$ have the same correlation structure as the one under the multinomial distribution, the covariance is estimated by

$$(12) \quad \widehat{\mathrm{Cov}}(\hat{M}_a, \hat{M}_{a'}) = -\sqrt{\frac{\hat{P}_a \hat{P}_{a'} \hat{V}(\hat{M}_a) \hat{V}(\hat{M}_{a'})}{(1 - \hat{P}_a)(1 - \hat{P}_{a'})}}$$

where P_a denotes the probability of landing at age a, that is $\hat{P}_a = \hat{M}_a/G$. G is the number of fish stocked. Because the covariances are expected to be negative, we get a conservative evaluation of $V(\hat{S})$ by assuming $\widehat{\mathrm{Cov}}(\hat{M}_a, \hat{M}_{a'}) = 0$. The recovery rate, the ratio of stocked fish recovered to the total number of fish stocked, denoted by $M./G$ is estimated by

$$(13) \quad \hat{Y}_A = \frac{\hat{M}.}{G}$$

where $M. = \sum_{a=0}^{A} M_a$. The variance estimates of $\hat{Y}_A$ is given by

$$(14) \quad \hat{V}(\hat{Y}_A) = \frac{1}{G^2} \hat{V}(\hat{M}.)$$

where $\hat{V}(\hat{M}.)$ is

$$\hat{V}(\hat{M}.) = \sum_{a=0}^{A} \hat{V}(\hat{M}_a) + 2 \sum_{a<a'}^{A} \widehat{\mathrm{Cov}}(\hat{M}_a, \hat{M}_{a'}).$$

Flounder in Fukushima Prefecture: A Case Study

Flounder is an important coastal fishery resource, and its annual catch in Japan was 5718 tons in 1989. The demand for flounder is high. As the annual catch of flounder has been

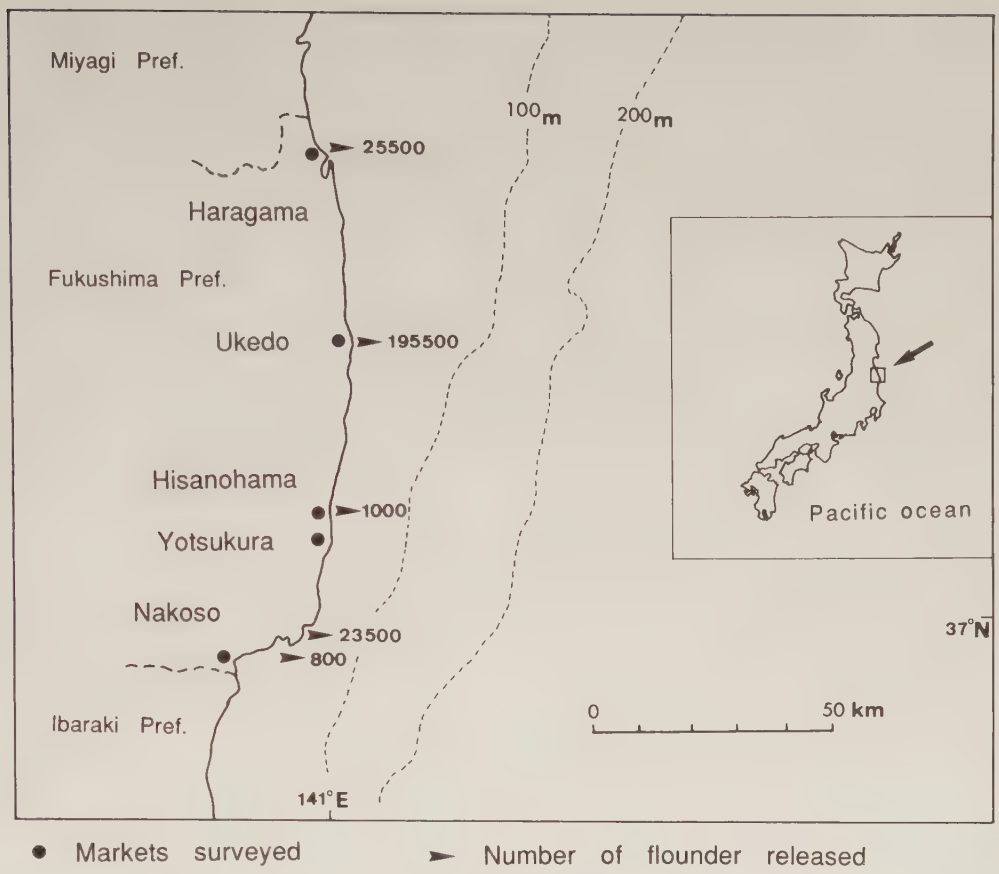

FIG. 1. Location of the markets surveyed in Fukushima Prefecture and release sites in 1987.

decreasing gradually, the price has become higher. Stock enhancement has been carried out for flounder since 1980. Flounder fingerlings are currently released in 30 prefectures, and the total number of released fish reached 11 million in 1989. It is one of the principal species targeted for stock enhancement programs in Japan.

Our case study deals with the flounder stock enhancement program in Fukushima Prefecture because the data were of good quality. The program was initiated in 1982. The annual number of fingerings released in this prefecture increased from 5000 in 1982 to 39 000 in 1986. In 1987, the prefecture enlarged the enhancement program, and the number of fingerlings released reached 246 000. The annual catch of flounder in Fukushima Prefecture has fluctuated between 88 and 669 tons during the last 15 yr (1973–87), with a mean value of 317 tons over the last 5 yr. The total landings for flounder in 1987 were valued at $7.41 million (U.S.). The unit market price at landing ranges from about 1000 yen ($8 (U.S.))/kg to 5000 yen/kg, with larger fish being more expensive.

We analyzed the data for the 1987 year-class surveyed from January 1988 to August 1990. The data were taken from the report of Fukushima Prefecture (Fukushima Prefectural Fisheries Experimental Station 1989, 1990, 1991). Fingerlings produced in tanks were released into the sea around Fukushima Prefecture in 1987 (Fig. 1). The fingerlings had hatched about 5 mo before release. The average total length of released fish was about 10 cm. Fish were released from August through October. Released fish had grown to about 20 cm in total length by January1988 when they began to be caught by trawlers. Offshore fishing grounds for trawlers cover a wide area, with the operation depth ranging from 20 to 150 m. Fish are also caught in gill nets close to shore at depths ranging from 10 to 50 m.

TABLE 2. Landings of flounder and landing days in each market of Fukushima Prefecture in 1987. G, gill net; T, trawl.

Market	No.	Fishing gear	Annual landing (kg)	Landing days
Shinchi	1	G	7 511	110
Haragama	2	G	38 336	156
	3[a]	T	63 047	116
Kashima	4	G	13 670	100
Ukedo	5[a]	G	28 443	127
Hisanohama	6	G	665	131
	7[a]	T	29 397	114
Yotsukura	8	G	1 765	33
	9[a]	T	30 504	187
Numanouchi	10	G	76	—
Ena	11	T	26 645	142
Onahama	12	G	1 864	54
	13	T	30 661	144
Nakoso	14[a]	T	23 152	142
Total (mean)			295 736	(120)

[a]Markets surveyed.

Flounder are landed at 14 commercial markets of fishermen's cooperative associations (Table 2). The landings are recorded by market and by type of gear. Five of the 14 markets were sampled, and a sample of 104 landing days was selected and measured from any chosen market. We analyzed these data through simple random sampling for both primary and secon

TABLE 3. Landing data for flounder (1987 year class) obtained in a survey of five selected fish markets in Fukushima Prefecture, conducted in 1988.

Market	Survey period	Survey days	Fishing gear	Sample size	Released fish in the sample	Mark ratio (%)
Haragama	Apr.–Nov.	46	Trawl	6 305	1 551	24.6
Ukedo	Jun.–Dec.	9	Gill net	950	580	61.1
Hisanohama	Jan.–Dec.	21	Trawl	1 136	411	36.2
Yotsukura	Jan.–Dec.	18	Trawl	1 354	264	19.5
Nakoso	Jan.–Dec.	10	Trawl	375	83	22.1
Total		104		10 120	2 889	28.5

TABLE 4. Estimates of M, N, and R and their variances for 14 markets in Fukushima Prefecture based on the data in Table 3.

	M	N	R
Estimate	50 978	152 076	0.335
95% C.I.	[23 860, 78 097]	[102 970, 201 183]	[0.199, 0.472]
W.Var.[a]	$6\,837 \times 10^4$	$29\,268 \times 10^4$	
B.Var.[b]	$12\,307 \times 10^4$	$33\,504 \times 10^4$	
Var.	$19\,144 \times 10^4$	$62\,772 \times 10^4$	4.9×10^{-3}

[a]First term of variance equations related to within-variance.
[b]Second term of variance equations related to between-variance.

dary units despite the fact that the sample was taken by purposive selection.

Released flounder were easily distinguished by the black pigment on their eyeless side. Pooling the data from the five markets resulted in a total of 16 565 flounder from the 1987 year-class. We identified 3575 released flounder in the landings. The data obtained during the 1988 survey are shown in Table 3.

Results and Discussion

As surveys were not performed in every month, we carried out the analysis only by year. First, we analyzed the survey data of 1988 (Table 3) to study sampling strategies.

The variance estimates for $\hat{M}$, $\hat{N}$, and $\hat{R}$ were quite large. The variances among markets were large and accounted for about 64% (for $\hat{M}$) and 53% (for $\hat{N}$) of the total variance, respectively (Table 4). To determine the number of primary and secondary units required for a predetermined error, we calculated the standard errors as functions of the sample sizes of primary and secondary units. We consider the variance equations (4) and (6) now with k and $d_{i(j)}$ as variables. The number of landing days was assumed to be 120, the mean value shown in Table 2, in each market. The contour lines of SD ($\hat{M}$) and SD($\hat{N}$) are shown in Fig. 2. This estimate was based on a simple random sampling from the total landing days, but the actual surveys stratified the days by month. Because we can expect that there is seasonal variation, the within-variance among days in each market was overestimated. Hence, the contour lines would be more vertical than those obtained assuming simple random sampling. Figure 2 shows that the variances would be reduced considerably if we added a few markets to the sample, while the number of survey days does not affect the accuracy of the estimation.

Variations among markets for both the total catch and the number of released fish are considerably larger than those among days within markets. It is therefore recommended to survey as many markets as possible at the expense of survey days. To see this, we consider the simple cost function

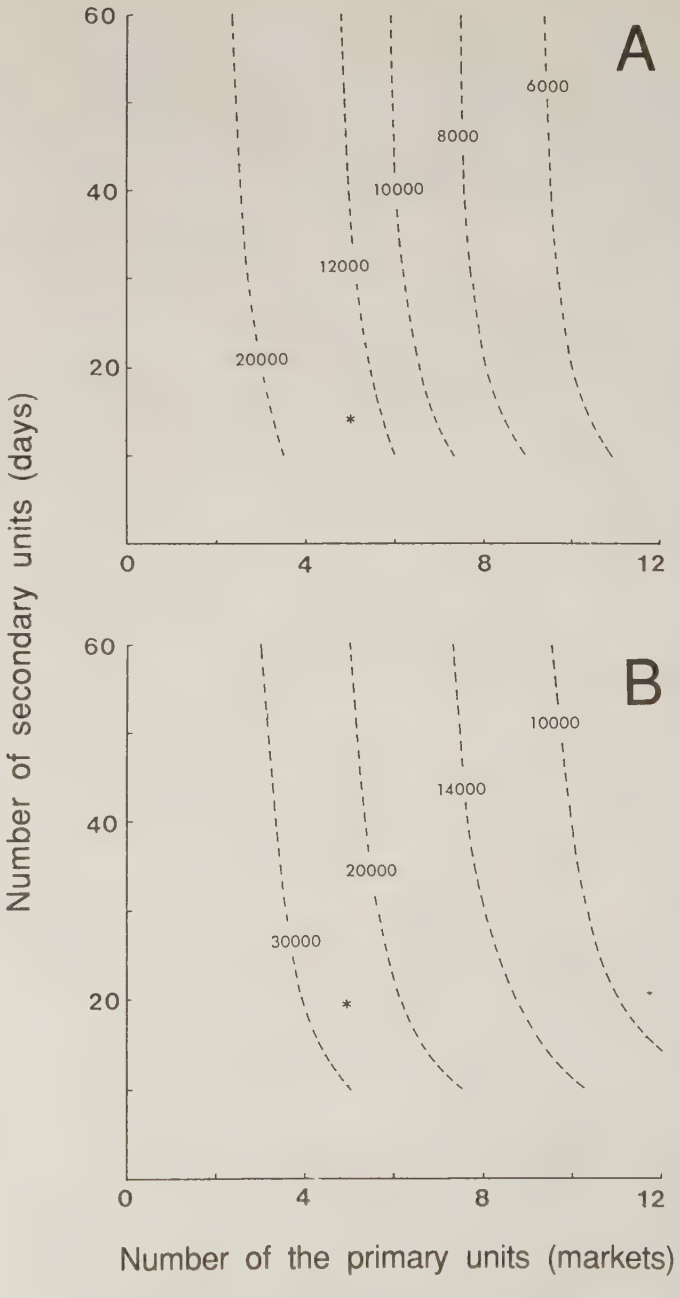

FIG. 2. Relation between the number of primary and secondary units for (A) the estimate of SD ($\hat{M}$) and (B) that of SD ($\hat{N}$) in Fukushima Prefecture. The asterisk shows the present value of the estimates of SD.

TABLE 5. Values for M, N, and R with their relative efficiencies otained using trawl landing data with no stratification performed and with stratification performed. When the stratification was performed, the subpopulation of six trawl markets was divided into two strata: Haragama and the other five markets.

	M	N	R
Not stratified			
Estimate	15 033	61 361	0.245
95% C.I.	[10 507, 19 559]	[41 712, 81 010]	[0.207, 0.283]
W.Var.[a]	$2\,668 \times 10^3$	$36\,206 \times 10^3$	
B.Var.[b]	$2\,665 \times 10^3$	$64\,297 \times 10^3$	
Var.	$5\,333 \times 10^3$	$100\,503 \times 10^3$	3.7×10^{-4}
Stratified			
Estimate	14 096	57 579	0.245
95% C.I.	[10 362, 17 830]	[39 845, 75 313]	[0.198, 0.292]
W.Var.[a]	$2\,761 \times 10^3$	$35\,677 \times 10^3$	
B.Var.[b]	868×10^3	$46\,192 \times 10^3$	
Var.ST	$3\,629 \times 10^3$	$81\,869 \times 10^3$	5.7×10^{-4}
Efficiency[c]	0.68	0.81	1.53

[a]First term of variance equations related to within-variance.
[b]Second term of variance equations related to between-variance.
[c]Var.ST/Var.

$$C = c_1 k + c_2 k d$$

where c_1 is the cost proportional to the number of markets and c_2 is the cost proportional to the total number of survey days. The optimum number of survey days to be sampled is given by

$$(15) \quad d = \sqrt{\frac{c_1}{c_2} \frac{\hat{\sigma}^2_{M_w}}{\hat{\sigma}^2_{M_b}}}$$

from equation (4); here,

$$\hat{\sigma}^2_{M_w} = \frac{1}{k} \sum_{j=1}^{k} D_{i(j)}{}^2 \hat{\sigma}^2_{M_{i(j)}}.$$

We obtained a value of 5.08 for $\hat{\sigma}^2_{M_w}/\hat{\sigma}^2_{M_b}$. The value for c_1/c_2 is unknown but it is thought to be large. The required number of survey days for a year is only 12.6 for $c_1/c_2 = 30$ (2.3 for $c_1/c_2 = 1$). Because a minimum of 2 d is required to provide an estimate of the variation between days within a market, a subsample of 2 d per market and month is recommended.

It was assumed that the large variances for $\hat{M}$ and $\hat{N}$ were the result of the different age selectivities of the fishing gear and different fishing efforts among markets. Therefore, stratification of markets by the type of fishing gear and catch quantities should be effective in reducing the variances among markets. To consider the efficiency of a stratification, we poststratified four trawl markets sampled into two strata according to annual catch in weight. Because the market selected was based on a gillnet fishery, we removed the stratum of gill nets because the variation among markets could not be calculated. The first stratum was Haragama market and the second stratum was composed of three other markets. The stratification was efficient in decreasing the variances among markets of $\hat{M}$ and $\hat{N}$ and was more effective for the estimate of M than for that of N. However, the value of efficiency for $\hat{R}$ was 1.53 from which we concluded that the stratification was not effective for the estimation of R. However, the variance was small and had little influence on the estimate of R (Table 5). The correlation between $\hat{M}_i$ and $\hat{N}_i$ is considered to relate the reducing efficiency

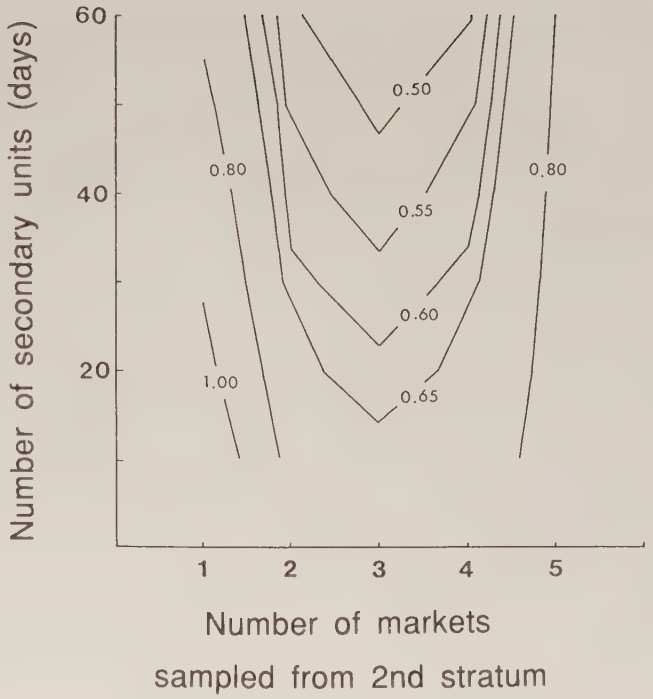

FIG. 3. Efficiency of stratification when Haragama market and three trawl markets are assumed to be sampled from each stratum. The contour line shows the value of $\hat{V}(\hat{M}_{ST})/\hat{V}(\hat{M})$.

of the stratification for $\hat{R}$. The stratification of the markets by landing weights may reduce the correlation that influences equation (10). The accuracy of $\hat{R}$ might have decreased because of the decrease of the correlation by the stratification.

The variance among markets of $\hat{M}$ was larger than that of $\hat{N}$. This can be related to the distribution of fish in the fishing grounds. Because most of the fingerlings were released at Ukedo (Fig. 1) the mark ratio obtained for this market was the largest among the markets surveyed (Table 3). This situation continued for the 3 yr surveyed after release, although the value of the ratio was decreasing year by year. Geographical stratification of markets, taking into account the number of fingerlings released and the number of release sites, is strongly recommended for the estimation of M. Variance among days within markets is thought to be caused by the seasonal variation in landings. For this reason, it is recommended that the analysis be carried out by month.

We calculated the efficiencies of stratification as $\hat{V}(\hat{M}_{ST})/\hat{V}(\hat{M})$ for several values of primary and secondary units. The efficiency decreased gradually when the first-stage sampling fraction became larger (Fig. 3). However, the estimates become more accurate as the number of markets selected increases.

To evaluate the effectiveness of our predictions for 3 yr after release, we assumed the same number of landing days for 1988, 1989, and 1990 in the five markets sampled. In the current survey, we intentionally selected five markets with large landings. To avoid overestimation, we removed from the population the markets of gillnetters (Shinchi, Hisanohama, Yotsukura, Numanouchi, and Onahama) whose annual landing weights were relatively small (Table 2). The population in the survey area (K) consisted of nine markets. The estimates of M, R, and S for 3 yr are shown in Table 6. The total number recovered with the 95% confidence interval was estimated as 36 982 [21 072, 52 892]. The estimate of the mark ratio decreased yearly. This may be because the survival rate of

TABLE 6. Estimates of M, R, and S (million yen) with 95% C.I. for nine markets in Fukushima Prefecture. C and W were mean unit prices (yen) and mean body weights (grams) used in the calculation.

	1988	1989	1990	Total
M	32 772	3 713	497	36 982
	[16 971, 48 572]	[1884, 5542]	[156, 838]	[21 073, 52 891]
R	0.335	0.170	0.037	—
	[0.216, 0.454]	[0.079, 0.264]	[0.004, 0.069]	—
S	19.66	10.40	2.49	32.55
	[10.18, 29.14]	[5.28, 15.52]	[0.78, 4.19]	[21.64, 43.45]
C	2 000	4 000	5 000	—
W	300	700	1 000	—

released fingerlings is smaller than that of wild flounder. As well, the immigration of wild flounder and the emmigration of released fish should occur. Fujita and Mizuno (1990) have stated that released flounder grow rapidly and many of them may have been caught before the recruitment of wild flounder. The estimate of recovery rate was 0.150 [0.086, 0.215]. The estimate of total income ($\hat{S}$) was 32.55 [21.64, 43.45] million yen ($260 [173, 348] thousand (U.S.)) under the average unit prices C_a and the estimate of average body weights W_a given in Table 6. Here, we calculated the variance of $\hat{S}$ assuming that W_a was known and $\widehat{\mathrm{Cov}}(M_a, M_{a'})$ were neglected. The average cost of a reared, 10-cm flounder is about 100 yen, including facilities and personnel costs. The profit of the stock enhancement program is estimated at 7.91 [−2.99, 18.82] million yen ($63[−24, 151] thousand (U.S.)). We believe there is still room for improvement in the cost. In the National Sea-Farming Center, Miyako Station, the cost of a 10-cm fingerling was 51 yen in 1987. Considering a cost of 50 yen, we obtained a profit of 20.23 [9.32, 31.14] million yen ($162 [75, 249] thousand (U.S.)). The estimate did not include the recoveries after September 1990. Hence the total recovery estimate should be considered conservative. We found that the stock enhancement program is economically profitable, although decreases in the cost of production of fingerlings and the fishing intensity for the age 1 fish should be expected in the future. In 1991, the Miyako station produced 2.5 million 3-cm flounder. Releases of a million flounder are feasible when the corresponding facilities and manpower are provided. We suggest that a comprehensive plan for stocking is appropriate, considering the population size of the wild stock and the actual condition of the fishery.

The stratified two-stage simple random sampling survey proposed in this paper is efficient for evaluating the stocking effectiveness and the number of fish in landings by age. We neglected the error that may be introduced by aging, although it may become larger for older age-classes. The method is applicable to any fish species. Hatcheries in Norway, the United States, and Japan have continued for many decades to produce and release larvae or fingerlings without adequate evaluation of the effectiveness. Techniques for producing fingerings have advanced rapidly in recent years. The current annual investment of the Japanese Central Government to the program has reached $40 million (U.S.). A scientific approach to measure the effectiveness of the program necessary for determining the future course of the enhancement program would seem essential.

Acknowledgements

We thank Mr. Tsuneo Fujita, Fukushima Prefectural Fisheries Experimental Station, for providing the data and helpful information. We also thank Dr. Luis A. Pastene, Ocean Research Institute, University of Tokyo, Dr. Jeffrey L. Thorne, Department of Plant Breeding and Biometry, Cornell University, and Dr. Byron Sigel, Embassy of the United States of America, for their useful comments on this manuscript. Comments from anonymous reviewers led to improvements in the manuscript. This work was supported by a contract between the Fishery Agency of Japan and the Japan Sea-Farming Association.

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Appendix: Derivation of Equation (5)

The following relation holds:

$$\text{(A.1)}\quad \frac{1}{n(n-1)}\sum_{i<j}^{n}(x_i-x_j)^2=\frac{1}{n-1}\sum_{i=1}^{n}(x_i-\bar{x})^2$$

where $\bar{x}=\sum_{i=1}^{n}x_i/n$. Suppose $(x_1, x_2, \ldots, x_n)$ is a random sample from the population $(X_1, X_2, \ldots, X_N)$:

$$E\left\{\frac{1}{n(n-1)}\sum_{i<j}^{n}(x_i-x_j)^2\right\}$$

$$=\frac{1}{n(n-1)}\sum_{i<j}^{N}(X_i-X_j)^2\frac{n(n-1)}{N(n-1)}$$

$$=\frac{1}{N(N-1)}\sum_{i<j}^{N}(X_i-X_j)^2$$

$$=\frac{1}{N-1}\sum_{i=1}^{N}(X_i-\bar{X})^2.$$

Let

$$s_x^2=\frac{1}{n-1}\sum_{i=1}^{n}(X_i-\bar{X})^2$$

$$\sigma_b^{*2}=\frac{1}{N-1}\sum_{i=1}^{N}(x_i-\bar{x})^2$$

and we have

$$\text{(A.2)}\quad E(s_x^2)=\sigma_b^{*2}.$$

In our problem, the between-variance

$$\text{(A.3)}\quad \sigma_{M_b}^{*2}=\frac{1}{K-1}\sum_{i=1}^{K}(M_i-\bar{M})^2$$

$$=\frac{1}{K(K-1)}\sum_{i<j}^{K}(M_i-M_j)^2$$

should be estimated unbiasedly, where $\bar{M}=M/K$. Let us consider the following expectation using (A.2), which can be calculated by two steps, E_1 and E_2, corresponding to the first and second sampling stages, respectively:

$$E\left\{\sum_{i(j)<i(l)}^{k}(\hat{M}_{i(j)}-\hat{M}_{i(l)})^2\right\}$$

$$=E_1E_2\left\{\sum_{i(j)<i(l)}^{k}[(\hat{M}_{i(j)}-M_{i(j)})-(\hat{M}_{i(l)}-M_{i(l)})+(M_{i(j)}-M_{i(l)})]^2\right\}$$

$$=E_1\left\{\sum_{i(j)<i(l)}^{k}\left[E_2(\hat{M}_{i(j)}-M_{i(j)})^2+E_2(\hat{M}_{i(l)}-M_{i(l)})^2+(M_{i(j)}-M_{i(l)})^2\right]\right\}$$

$$=E_1\left\{\sum_{i(j)<i(l)}^{k}\left[D_{i(j)}^2\frac{D_{i(j)}-d_{i(j)}}{D_{i(j)}-1}\frac{\sigma_{i(j)}^2}{d_{i(j)}}+D_{i(l)}^2\frac{D_{i(l)}-d_{i(l)}}{D_{i(l)}-1}\frac{\sigma_{i(l)}^2}{d_{i(l)}}+(M_{i(j)}-M_{i(l)})^2\right]\right\}$$

$$=\frac{k(k-1)}{K(K-1)}\left\{\sum_{i<j}^{K}\left(D_i^2\frac{D_i-d_i}{D_i-1}\frac{\sigma_i^2}{d_i}+D_j^2\frac{D_j-d_j}{D_j-1}\frac{\sigma_j^2}{d_j}\right)+\sum_{i<j}^{K}(M_i-M_j)^2\right\}$$

$$=\frac{k(k-1)}{K(K-1)}\left\{(K-1)\sum_{i=1}^{K}D_i^2\frac{D_i-d_i}{D_i-1}\frac{\sigma_i^2}{d_i}+\sum_{i<j}^{K}(M_i-M_j)^2\right\}$$

$$=k(k-1)\left\{\frac{1}{K}\sum_{i}^{K}D_i^2\frac{D_i-d_i}{D_i-1}\frac{\sigma_i^2}{d_i}+\frac{1}{K-1}\sum_{i=1}^{K}(M_i-\bar{M})^2\right\}$$

where E denotes expected value over all samples, E_2 denotes averaging over all possible second-stage selections from a fixed set of units, and E_1 denotes averaging over all first-stage selections.

The unbiased estimator of $\sigma^{*2}_{M_b}$ is therefore given by

$$\hat{\sigma}^{*2}_{M_b} = \frac{1}{k(k-1)} \sum_{i(j)<i(l)}^{k} (\hat{M}_{i(j)} - \hat{M}_{i(l)})^2 - \frac{1}{k} \sum_{j=1}^{k} D^2_{i(j)} \frac{D_{i(j)} - d_{i(j)}}{D_{i(j)} - 1} \frac{\hat{\sigma}^2_{i(j)}}{d_{i(j)}}$$

$$= \frac{1}{k-1} \sum_{j=1}^{k} (\hat{M}_{i(j)} - \tilde{M})^2 - \frac{1}{k} \sum_{j=1}^{k} D^2_{i(j)} \frac{D_{i(j)} - d_{i(j)}}{D_{i(j)} - 1} \frac{\hat{\sigma}^2_{i(j)}}{d_{i(j)}}$$

where $\tilde{M} = \frac{1}{k} \sum_{j=1}^{k} \hat{M}_{i(j)}$. Finally, since

$$\text{(A.4)} \quad \hat{\sigma}^2_{M_b} = \frac{k-1}{k} \hat{\sigma}^{*2}_{M_b}$$

we have

$$\text{(A.5)} \quad \hat{\sigma}^2_{M_b} = \frac{1}{k} \sum_{j=1}^{k} (\hat{M}_{i(j)} - \tilde{M})^2 - \frac{(k-1)}{k^2} \sum_{j=1}^{k} D^2_{i(j)} \frac{D_{i(j)} - d_{i(j)}}{D_{i(j)} - 1} \frac{\hat{\sigma}^2_{i(j)}}{d_{i(j)}}.$$

Dynamics of Energy Storage in a Freshwater Predator (*Nephelopsis obscura*) following Winter Stresses

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Reddy, D. C., E. Dratnal, and R. W. Davies. 1992. Dynamics of energy storage in a freshwater predator (*Nephelopsis obscura*) following winter stresses. Can. J. Fish. Aquat. Sci. 49: 1583–1587.

Two groups of *Nephelopsis obscura* were maintained under identical conditions (20°C, 100% dissolved oxygen, 12 h light : 12 h dark regime, ad libitum food) from post-hatchling size (10–11 mg) to sexual maturity (500–700 mg). One group (winter *N. obscura*) had overwintered in the field and degrown to 10–11 mg, while the other group (summer *N. obscura*) had hatched in the laboratory. The winter *N. obscura* at post-hatchling size had significantly lower accumulations of total lipid (3.9%), total protein (50.7%), free amino acids (1.6%), and glycogen (3.4%) than summer leeches, indicating that winter stresses (low water temperatures, low dissolved oxygen, restricted prey availability) had reduced energy stores and caused degrowth. The cryoprotective glycerol concentration, however, was significantly higher (1.9%) in winter leeches. At or just prior to sexual maturity, both winter and summer leeches showed decreases in total protein, glycogen, and total lipid which were attributed to the increased energetic demands of gametogenesis and maturation. The effects of, and compensation for, winter stresses were evident throughout the development of the winter leeches which exhibited enhanced energy allocation to storage compared with summer leeches.

Deux groupes de *Nephelopsis obscura* ont été détenus dans des conditions identiques (20 °C, 100 % d'oxygène dissous, photopériode 12 h : 12 h, alimentation à volonté) de la taille post-larvaire (10–11 mg) à la maturité sexuelle (500–700 mg). Un groupe (*N. obscura* d'hiver) avait passé l'hiver dans leur milieu naturel et leur poids avait diminué pour atteindre 10–11 mg, tandis que l'autre groupe (*N. obscura* d'été) avait éclos en laboratoire. Chez les *N. obscura* d'hiver post-larvaires, l'accumulation de lipides totaux (3,9 %), de protéines totales (50,7 %) d'acides aminés libres (1,6 %) et de glycogène (3,4 %) était inférieure à celle des sangsues d'été, ce qui indique que les stress de l'hiver (basses températures de l'eau, faible teneur en oxygène dissous, disponibilité réduite des proies) ont réduit les réserves énergétiques et ont causé une décroissance. Toutefois, la concentration du glycérol cryoprotecteur était significativement plus élevée chez les sangsues d'hiver. Au moment de la maturité sexuelle ou juste avant, les sangsues d'été et d'hiver ont montré une diminution des quantités de protéines totales, de glycogène et des lipides totaux, laquelle était imputable à des demandes énergétiques accrues durant la gamétogénèse et la maturation. Les effets des agressions hivernales et les mesures compensatoires ont été évidents durant tout le développement des sangsues d'hiver, chez lesquelles les dépenses d'énergie pour le stockage étaient supérieures à celles des sangsues d'été.

Received October 1, 1991
Accepted February 10, 1992
(JB246)

Reçu le 1 octobre 1991
Accepté le 10 février 1992

Freshwater invertebrates in temperate zones are usually subjected to seasonal periods of low water temperatures, low dissolved oxygen concentrations, and restricted food availability. During winter, animals frequently have to rely on energy stored during periods favourable to energy acquisition (Boddington and Mettrick 1971; Streit 1978; Meyer 1990), although energetic demands are often mitigated by decreased activity. How animals apportion their energy to growth, reproduction, storage, and activity at different life-history stages directly affects ecological success and fitness (Calow 1977). In many animals, an increased proportion of assimilated food is allocated to reserves rather than to immediate use to ameliorate temporal mismatches between food supply and energy demand (Larson 1991).

This is also the case in *Nephelopsis obscura*, a predatory leech widely distributed in lentic freshwater ecosystems of Canada and the northern parts of the United States (Davies 1991). Over its geographic range, *N. obscura* shows considerable variation in both body size at maturity and life-history traits (Davies and Everett 1977; Peterson 1983; Baird et el. 1986, 1987b; Linton and Davies 1987). In southern Alberta, lakes are ice covered for 90–120 d during winter (Baird et al. 1987a), and *N. obscura* exhibits degrowth during these times (Davies and Everett 1977; Dratnal and Davies 1990). Post-hatchling-sized individuals that have overwintered have been shown to differ from similarly sized individuals hatched in the summer with respect to ecophysiological processes and life-history and fitness characteristics (Dratnal and Davies 1990). Overwintered *N. obscura* had only half the total lipid concentration of similarly sized individuals hatched in the summer. However, under optimal feeding conditions, lipid depletion at maturity was fully compensated for in overwintered leeches through increased food ingestion, higher absorption efficiency, and the allocation of more energy to lipid accumulation than other growth functions.

Little is known of the influence of overwintering on the metabolic responses of *N. obscura* throughout its life history. The adaptiveness of these physiological responses is also unknown. We therefore carried out experiments to determine whether or not differences occur in the pattern of energy-reserve accu

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mulation at different life-history stages between two groups of *N. obscura* reared under identical conditions. One group (called winter *N. obscura*) had overwintered in the field; the other group of the same weight had hatched in the laboratory from cocoons produced in early summer (called summer *N. obscura*). As summer *N. obscura* were maintained in constant conditions over their entire life history, they were considered controls in the assessment of the changes in energy reserve deposition, accumulation, and utilization exhibited by winter *N. obscura*.

Materials and Methods

Early in May 1990, post-hatchling (8–10 mg) *N. obscura* that had overwintered in the field (winter *N. obscura*) were collected from Stephenson's Pond, a small (2.2 ha) prairie pothole pond 5 km northwest of Calgary, Alberta. They were immediately transferred to six large laboratory holding tanks with filtered, air-saturated (100%) pond water and kept for 2 d at 5 ± 1°C under a 12 h light : 12 h dark regime without food before a set was randomly selected for the first measurements. The remaining leeches were gradually acclimated to 20°C (1°C increase·d^{-1}), 100% oxygen saturation, and a 12 h light : 12 h dark regime. Water in the acclimatization tanks was continuously aerated and changed once a week. Leech biomass never exceeded 1 g·L^{-1}. In July 1990, *N. obscura* cocoons were collected from Stephenson's Pond (water temperature 20°C) immediately after deposition and maintained in the laboratory in filtered, aerated pond water at 20°C for approximately 4 wk until they hatched. After hatching, *N. obscura* in the 9–11 mg range (summer *N. obscura*) were reared in large laboratory holding tanks under conditions identical to the winter *N. obscura*.

Both winter and summer *N. obscura* were fed *T. tubifex* ad libitum three times a week for a 2-h period. To ensure a shortage-free food supply, ingestion was tested for an asymptotic level of feeding (Dratnal and Davies 1990). Leech wet weights (WWT) were measured every week with the difference between successive weighings considered growth. Subsets of leeches (n = 20 for each group) were maintained separately in 70-cm^2 containers to assess growth and the onset of sexual maturity as determined by clitellum development.

Both winter and summer *N. obscura* exhibited sigmoid growth, and six representative life-history stages were selected, characterized by different growth rates (thus varying energy allocation to growth), and by different stages of sexual maturity: (1) post-hatchling size (9.9–11.1 mg), (2) initially accelerating, relatively flat growth, (3) late accelerating growth, (4) inflection point of growth curve, (5) appearance of clitellum indicating sexual maturity, and (6) decelerating growth in fully mature animals (Table 1). At each stage, total protein (TP), total lipid (TL), glycogen (GN), glycerol (GL), free amino acids (FAA), and active phosphorylase (PHO-A) were measured to determine if differences occurred in the pattern of reserve deposition, accumulation, and utilization. Differences between the two groups of leeches reflect differences in metabolic responses due to differences in prehistory, while differences between stages within a group reflect differences in metabolic responses throughout the life history.

Because these determinations are destructive, parallel sets of winter (n = 30) and summer (n = 30) *N. obscura* were randomly selected at each time of measurement and sacrificed to measure the variables (n = 5 for each variable). At each stage, winter and summer *N. obscura* were weighed separately (WWT) and either fixed in liquid nitrogen using quick-freeze tongs (Bergmeyer 1974) for measurements of GN, GL, FAA, and PHO-A or freeze-dried to a constant weight for measurements of TL and TP. The frozen leeches were immediately powdered in a mortar constantly cooled with dry ice, mixed with approximately four volumes of ice-cold 0.6 N perchloric acid (PCA), homogenized, centrifuged at 0–4°C, and the extracts stored at −20°C before being assayed for GN and GL following Keppler and Decker (1974) and Wieland (1974), respectively. Frozen leeches ground to a fine powder were homogenized in 1:1 0.1 M sodium fluoride (pH 6.5) and 0.037 M ethylenediaminetetraacetic acid (pH 6.5) medium, centrifuged, and the resultant supernatants used to measure PHO-A (EC 2.4.1.1) in the direction of GN synthesis (Cori et al. 1955) by determining the amount of inorganic phosphate (Pi) (Fiske and Subba Row 1925) liberated from glucose-1-phosphate in the presence of GN as primer. FAA were measured in 10% trichloroacetic acid extracts of frozen leeches following Colowick and Kaplan (1957). TL and TP (dry weight (DWT)) were measured following Folch et al. (1957) and Bradford (1976), respectively. The results are presented as concentrations (milligrams per 100 mg dry weight) or activity (micromoles Pi per milligram TP per hour).

Net relative accumulation (NRA) was calculated as

$$\mathrm{NRA} = \frac{(P_{t1} - P_{t0})/P_{t0}}{(M_{t1} - M_{t0})/M_{t0}}$$

where P is the total mass (milligrams) of biochemical component, M is leech DWT (milligrams), and $t0$ and $t1$ are two successive life-history stages. With $M_{t1} - M_{t0} > 0$, a NRA of 1 indicates that the net accumulation of a biochemical component is isometric with increases in body DWT, while a NRA of 0 indicates no accumulation.

Differences in the concentrations/activity of the biochemical components between the corresponding stages of winter and summer *N. obscura* were assessed using two-way ANOVA with alpha = 0.05.

Results

WWT of both winter and summer *N. obscura* were similar in stages 1–3. However, by stage 4, summer *N. obscura* were significantly heavier (Table 1). Sexual maturity, determined by the presence of a clitellum (stage 5), developed after 117–121 d.

Winter and summer *N. obscura* showed increases in TL concentration until stage 5 and a significant decrease after attaining sexual maturity (Fig. 1). Stage 1 and 2 winter *N. obscura* had only about half the TL concentration of summer leeches in the same stages. An ANOVA comparing stages 3–6 indicated that winter leeches had significantly higher TL than summer leeches.

Stage 1 and 2 winter *N. obscura* had significantly lower TP than summer *N. obscura*. An ANOVA comparing stages 3–6 indicated that winter *N. obscura* continued to have lower TP than summer *N. obscura*, although there was a significant interaction due to the disproportionate increase in TP in stage 4 winter leeches (Fig. 1).

In stage 1, winter *N. obscura* had 1.5 times more GL than summer *N. obscura*. From stages 2 through 6 the GL concentrations in winter *N. obscura* were significantly higher than those in summer *N. obscura*. Both groups showed significant increases in GL between stages 5 and 6 (Fig. 1).

TABLE 1. Mean (± SD) (n = 30) wet weights (mg) of winter and summer *Nephelopsis obscura* at each of the six life history stages at which biochemical components were measured. *Significant difference ($p < 0.05$) between pairs.

Leech group	Stage					
	1	2	3	4	5	6
Winter	9.9 ± 0.55	21.9 ± 0.56	165.4 ± 3.27	279.1 ± 5.31 *	349.3 ± 5.22 *	515.3 ± 13.59 *
Summer	11.1 ± 0.44	23.1 ± 0.36	163.1 ± 1.71	297.4 ± 2.23	478.7 ± 5.57	704.2 ± 10.71

Winter and summer *N. obscura* showed increases in FAA concentrations throughout their life history, and between stages 1 and 2, changes in FAA showed similar trends in both groups. Winter *N. obscura* had slightly more than half the FAA of summer *N. obscura* in stage 1. ANOVA comparing stages 2–6 indicated that FAA concentrations were significantly lower in winter *N. obscura* (Fig. 1).

Both groups of *N. obscura* showed increases in GN concentrations until stage 4 followed by a significant decrease thereafter (Fig. 1). Although winter *N. obscura* in stage 1 had only 75% of the GN concentration of summer *N. obscura*, GN concentrations in the remaining stages were not significantly different (Fig. 1).

Increases in PHO-A activity occurred throughout the life history of winter and summer leeches, with winter *N. obscura* having significantly lower PHO-A than summer *N. obscura* (Fig. 1).

NRA was calculated for five parameters: TP, primarily a structural body component; TL and GN, direct energy resources; FAA, catabolic products of TP; and GL, a product of lipolysis. Winter and summer *N. obscura* showed positive values of NRA over their entire life histories. However, NRA was much closer to being isometric with changes in body weight (DWT) in summer leeches. Winter leeches showed periods of enhanced accumulation, especially between the early stages. At maturity or immediately before maturity, both groups of leeches showed decreases in NRA of TP, TL, GN, and FAA but an increase in NRA of GL.

Discussion

The results show physiological differences between winter and summer *N. obscura* throughout their life history. These differences can be viewed, at least in part, as the result of the stress induced by winter environmental conditions.

Seasonal changes in energy reserves and utilization have been recorded in several groups of freshwater invertebrates, e.g. triclads (Boddington and Mettrick 1971), lamellibranchs (Streit 1978), rotifers (Guisande and Serrano 1989), cladocerans (Goulden and Place 1990), amphipods (Quigley et al. 1989), and stonefly larvae (Meyer 1990). The function of the allocation of assimilated energy to reserves rather than immediate use can be deduced from the timing of the reserve accumulation and its subsequent utilization relative to changes in the environment and physiological demands.

The recorded changes in the accumulation of body component variables of *N. obscura* (Fig. 1) reflect the dynamic interaction between deposition and utilization. Changes in the relative accumulation of energy reserves result from changes in the processes of deposition and utilization. Increased accumulation indicates either increased deposition, decreased utilization, or a combination of both. In this study the total amount of each variable always increased between successive life-history stages (NRA > 0). Thus, the energy reserve components were continuously deposited throughout the life history, but accumulation and utilization changed measurably.

Although *N. obscura* encounter reduced prey abundance (Davies et al. 1981, 1982) coupled with low temperatures and low dissolved oxygen concentrations (Baird et al. 1987a) during winter, they survive these winter stresses to reproduce the following summer (Davies and Everett 1977). During winter, *N. obscura* utilize stored energy for maintenance, resulting in lower TL, TP, and GN concentrations. Animals generally utilize TL and GN as energy resources, but under prolonged stress, TP is also used. The lower accumulation of FAA in winter leeches indicates the utilization of TP for maintenance.

Post-hatchling-sized winter *N. obscura* started with significantly lower TL, TP, and GN concentrations than similarly sized summer leeches. TL and GN reserves were quickly replenished and TL concentrations even slightly exceeded those of summer leeches. TP concentrations did not return to the level of summer *N. obscura*. Allocation of energy primarily towards storage reserves during periods favourable to energy acquisition emphasizes the importance of energy reserve deposition (King and Murphy 1985). Attaining a large body size as rapidly as possible is usually considered to increase fitness (Sibly and Calow 1986). Energy storage is directly related to survivorship and reproductive success, two important measures of fitness. This is especially true in semelparous organisms which, in low energy resource environments, supplement reproduction by energy from parental stores (Woolhead and Calow 1979). As both winter and summer *N. obscura* were fed prey ad libitum over the entire experimental period, it appears that the differences in energy reserve accumulation are the result of winter stresses tuning energy allocation so that as much energy as possible is allocated to storage to enhance survivorship in an unpredictable and/or variable environment. Energy is most frequently stored as TL because lipids contain at least twice the weight-specific energy content of carbohydrates or proteins (Hadley 1985).

The decreases in TP, GN, and TL between stages 5 and 6 can be attributed to increased energetic demands of sexual maturation and clitellum production. A decline in energy reserves during reproductive activity has been reported in aquatic invertebrates and vertebrates (Fitzpatrick 1976; Quigley et al. 1989; Larson 1991). Both winter and summer *N. obscura* follow a common pattern with regard to energy balance at or directly prior to attaining sexual maturity. GN is the first substrate used for production of metabolic energy during this period, followed by TL. Because TP is a major structural component of the body, a decrease in NRA results in decelerating growth rates in mature animals.

GL protects animal tissues from freezing by reducing the freezing point of the haemolymph and cell water (Siegert 1986). Although winter *N. obscura* had significantly higher GL concentrations than summer *N. obscura* following acclimation to 20°C, they gradually decreased their GL concentration as shown in insects by Chen and Denlinger (1990). Both winter and sum-

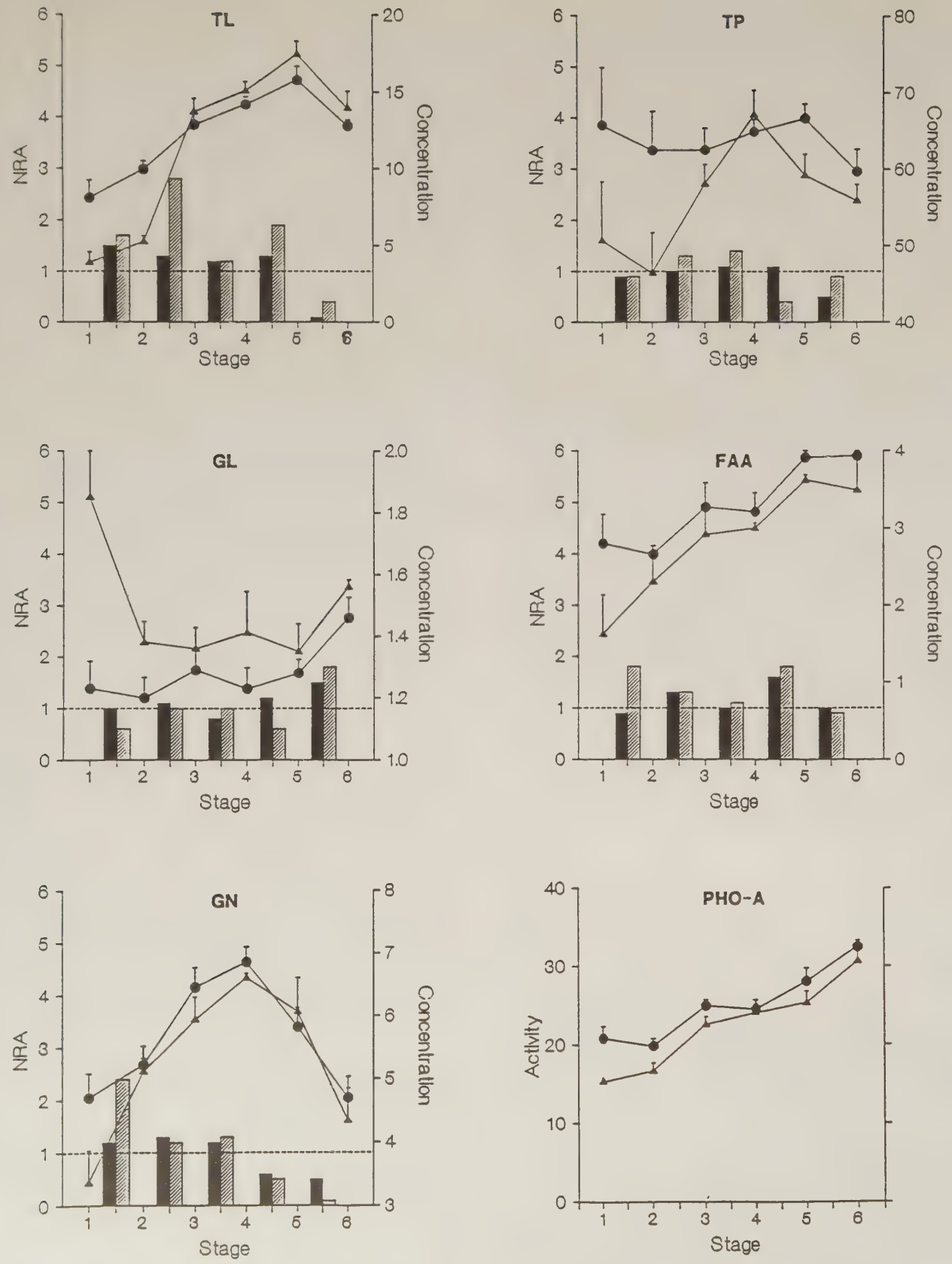

FIG. 1. Mean (±SD) (n = 5) body component concentrations (mg·100 mg dry weight^{-1})/activity (μmol Pi·mg TP^{-1}·h^{-1}) (triangles, winter; circles, summer) and the net relative accumulations (NRA) (open bars, winter; hatched bars, summer) in and between successive life-history stages of summer and winter *Nephelopsis obscura* (TL, total lipid; TP, total protein; GL, glycerol; FAA, free amino acids; GN, glycogen; PHO-A, active phosphorylase).

mer *N. obscura* significantly increased GL between stages 5 and 6, indicating enhanced production of energy through lipolysis as shown by the decreases in TL.

PHO-A breaks down GN, reflecting the rate at which GN is channelled into metabolic pathways to produce energy. Prolonged cold stress is known to increase GL concentrations which inhibit PHO-A activity (Siegert 1986). This was observed in stage 1 winter *N. obscura*. No significant change in PHO-A activity coupled with a significant increase in GN deposition between stages 1 and 2 in winter *N. obscura* indicates high GN deposition without increased utilization. In contrast, summer *N. obscura* during this period showed significantly higher PHO-A activity (Fig. 1), indicating increased GN utilization resulting in decreased accumulation. Both groups showed significant increases in PHO-A activity between stages 4 and 6, indicating a shift towards increased GN utilization to meet the energetic demands of maturation and sexual reproduction.

Positive values of NRA over the life history of both groups show that under optimal resource conditions, reserve utilization never exceeds deposition. Summer leeches allocated energy to storage approximately isometrically with the increase in body DWT except during the period of sexual maturation. In contrast, winter leeches with depleted storage reserves in stage 1 compensated for winter stresses by enhanced energy allocation to GN, TL, and TP during the early life-history stages, as demonstrated by NRA values >1.

Although these experiments and those of Dratnal and Davies (1990) both examine the effects of winter stresses on energy allocation and storage in *N. obscura* the results of the two studies are not directly comparable. In this study the physiological–biochemical adaptations of *N. obscura* to winter stresses were examined excluding possible foraging behaviour compensation by providing prey directly available to the leeches. In contrast, Dratnal and Davies (1990) provided prey with a soft mud substratum allowing prey antipredatory responses. *Nephelopsis obscura* feeding directly on prey showed higher feeding rate, higher growth rate, and a larger size at maturity than those feeding with a foraging constraint (Dratnal et al. 1992).

In summary, winter stresses that resulted in degrowth of *N. obscura* also resulted in significant reductions in TL, TP, and GN. Under optimal conditions, winter leeches rapidly made up these losses (NRA >1) and by stage 4 (the inflection point of the growth curve) had levels comparable with those of summer leeches. By maturity (stage 5), both the stressed winter *N. obscura* and the nonstressed summer *N. obscura* were almost equally prepared for the costs of reproduction.

Acknowledgements

It is a pleasure to acknowledge the technical assistance of B. Biernacka and to thank Dr. L. R. Linton for his comments and help with the statistical analyses.

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Trophic Level Relationships in Pelagic Food Webs: Comparisons Derived from Long-Term Data Sets for Oneida Lake, New York (USA), and Lake St. George, Ontario (Canada)

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McQueen, D. J., E. L. Mills, J. L. Forney, M. R. S. Johannes, and J. R. Post. 1992. Trophic level relationships in pelagic food webs: comparisons derived from long-term data sets for Oneida Lake, New York (USA), and Lake St. George, Ontario (Canada). Can. J. Fish. Aquat. Sci. 49: 1588–1596.

We used standardized methods to analyze a 14-yr data set from Oneida Lake and a 10-yr data set from Lake St. George. We estimated mean summer concentrations of several trophic level indicators including piscivores, planktivores, zooplankton, phytoplankton, and total phosphorus, and we then investigated the relationships between these variables. Both data sets yielded similar long-term and short-term trends. The long-term mean annual trends were that (1) the relationships between concentrations of planktivores and zooplankton (including daphnids) were always negative, (2) the relationships between concentrations of zooplankton and various measures of phytoplankton abundance were unpredictable and never statistically significant, and (3) the relationships between total phosphorus and various measures of phytoplankton abundance were always positive. Over short periods, the data from both lakes showed periodic, strong top-down relationships between concentrations of zooplankton (especially large *Daphnia*) and chlorophyll *a*, but these events were unpredictable and were seldom related to piscivore abundance.

Nous avons utilisé des méthodes normalisées pour analyser un ensemble de données sur 14 ans provenant du lac Oneida et un ensemble de données sur 10 ans provenant du lac St. George. Nous avons estimé les concentrations estivales moyennes de plusieurs indicateurs des niveaux trophiques, incluant les ichtyophages, les planctonophages, le zooplancton, le phytoplancton et le phosphore total et avons ensuite analysé les relations entre ces variables. Les deux ensembles de données ont montré des tendances similaires à long et à court terme. Les tendances annuelles moyennes à long terme étaient les suivantes : 1) les relations entre les concentrations de planctonophages et celles du zooplancton (incluant les daphnies) étaient toujours négatives, 2) les relations entre les concentrations du zooplancton et les différentes mesures de l'abondance du plancton étaient imprévisibles et jamais statistiquement significatives et 3) les relations entre le phosphore total et les différentes mesures de l'abondance du phytoplancton étaient toujours positives. Sur des périodes brèves, les données des deux lacs montraient des relations descendantes périodiques et fortes entre les concentrations de zooplancton (en particulier les daphnies de grande taille) et de chlorophylle *a*, mais ces événements étaient imprévisibles et étaient rarement reliés à l'abondance des ichtyophages.

Received October 8, 1991
Accepted February 7, 1992
(JB254)

Reçu le 8 octobre 1991
Accepté le 7 février 1992

Several general hypotheses have been proposed to predict relative impacts of predators and resource availability on trophic level biomass or productivity. The common theme of these top-down models (the biomanipulation model, Shapiro et al. 1982; the cascading model, Carpenter et al. 1985; and the holistic model, Persson et al. 1988) is that freshwater pelagic trophic levels are tightly linked so that changes in the fish communities cascade (Carpenter et al. 1985) down through the food web to influence primary producers and even nutrient availability.

A number of short-term biomanipulation studies on lakes, ponds, and enclosures support this view of trophic level relatedness (Hrbáček 1962; Lynch and Shapiro 1981; Osgood 1983; Shapiro and Wright 1984; Bergquist and Carpenter 1986; reviewed by De Melo et al. 1992). However, other studies (Benndorf et al. 1984, 1988; Lammens 1988; McQueen et al. 1989; reviewed in De Melo et al. 1992) have not reported strong links between fish and phytoplankton biomass. Most notable among these studies are the longer-term, whole-lake studies (Gophen 1984, 1985; Gophen et al. 1990; Benndorf et al. 1984, 1988; Mills et al. 1987; Lammens 1988; McQueen et al. 1989). It seems that the longer the study the more difficult it is to demonstrate strong links between all trophic levels.

The theme of the analysis that follows is to use two of these data sets to assess long-term trends. We expect that short-term responses to strong treatment effects will certainly demonstrate the transient influences of one community component on another (Kitchell et al. 1988). But are these effects sustainable

from year to year? Can we alter fish populations enough to create annual, sustained, cascading impacts on phytoplankton biomass and water clarity? We recognize that such complex questions will not be answered by one analysis, but our contribution can be to compare the relative magnitudes of various trophic level interactions observed over long periods (14 yr from Oneida Lake and 10 yr from Lake St. George) and to investigate these relationships in the context of the substantial changes that were observed in fish community structure.

Study Areas and Data Sets

Previously published portions of the Oneida Lake and Lake St. George data sets have yielded conflicting results. Data from Lake St. George (McQueen et al. 1989, 1990) suggested that zooplankton (including daphnids) had no long-term interannual impacts on phytoplankton biomass. Portions of the Oneida Lake data set (Mills et al. 1987) also suggested that top-down impacts were dampened at the bottom of the food web, but when the data set was restricted to include only years when *Daphnia pulex* was abundant, there was a significant top-down effect of daphnids on the concentration of chlorophyll *a*. In the analysis that follows, we have compared the patterns in these two data sets. Additionally, we have added three more years to the Lake St. George data set which now spans 1980–89, and we have completed the Oneida Lake data set by including all data for all years from 1975 to 1988.

Materials and Methods

Lake St. George is situated in southern Ontario, Canada. It is a kettle lake with a surface area of 10.4 ha, a maximum depth of 16 m, and a mean depth of 4.9 m. It is mesoeutrophic, has a clinograde oxygen profile, and is ice covered from approximately December through March. The fish community comprises 14 species. The dominant piscivores are northern pike (*Esox lucius*), largemouth bass (*Micropterus salmoides*), and walleye (*Stizostedion vitreum*), and the most abundant facultative and obligate planktivores are yellow perch (*Perca flavescens*), golden shiner (*Notemigonus crysoleucas*), and bluegill (*Lepomis macrochirus*).

Oneida Lake is a shallow, eutrophic lake (maximum depth 15 m, mean depth 6.8 m) covering 20 700 ha on the Ontario Lake Plain of central New York State. The lake is usually isothermal, but temporary stratification may develop during periods of extended calm weather. Ice cover is from approximately December through March. Over 60 fish species inhabit the lake. The walleye is the most abundant predator and walleye predation controls recruitment of yellow perch and strongly influences species and size composition of the fish community. Walleye and yellow perch share the limnetic region with two serranids, white perch (*Morone americana*) and white bass (*Morone chrysops*), and a clupeid, gizzard shad (*Dorosoma cepedianum*).

At Oneida Lake the zooplankton, phytoplankton, and water chemistry data were collected weekly, usually at five stations, from May through August 1975–88. Zooplankton density was estimated from vertical hauls with a 0.5-m-diameter, 153-μm-mesh net. Zooplankton were preserved in either 100% isopropyl alcohol or in sugar formalin, identified to species in three 1-mL aliquots, and enumerated manually or by computer (Mills and Confer 1986). Dry weight conversion factors for *Daphnia* and other taxa (Chamberlain 1975) were used to estimate zooplankton biomass. Samples for chlorophyll *a* and water chemistry were collected using 2-cm-i.d. tygon tubing stretched from the surface to 0.5 m off the bottom. Pigment analyses including phaeophytin were done using the method of Strickland and Parsons (1972), and total phosphorus was done according to Menzel and Corwin (1965).

The walleye was the most abundant piscivorous species, and age 0 fish were the predominant planktivores in Oneida Lake (Mills et al. 1987). Biomass of age 4 and older walleye in April was estimated by mark–recapture from 1957 through 1977, and after 1977, biomass was indexed from catches in the trapnets set by the Oneida Hatchery (Forney 1977). Planktivore biomass was estimated from the catch in bottom trawls. Trawls were fished at 10 sites at weekly intervals in July through mid-October and abundances were estimated from the mean catch and the area swept by the trawls (Forney 1971).

At Lake St. George, all of the zooplankton, phytoplankton, and water chemistry data were collected on a weekly or biweekly basis from May through August, 1980–89. The fish data were collected in the fall of each year using mark–recapture methods. Detailed descriptions of sampling methods, sample sizes, and estimates of errors are given in McQueen et al. (1986, 1989) and Johannes et al. (1989). Briefly, the zooplankton samples were taken at 1, 3, 5, 8, 12, and 14 m using a 35-L Plexiglas plankton trap (80-μm mesh) and combined samples were counted using a computer assisted calliper device (Sprules et al. 1981) that allowed the estimation of biomass. Chlorophyll *a* and water chemistry data were collected from the epilimnion using an integrated sampler. Analyses were carried out at the Canada Centre for Inland Waters using the methods of Aspila et al. (1976). Fish were marked and recaptured using the sequential methods described by Ricker (1975). In each year the experiment was continued until we had marked >5% of the estimated population.

Statistical analysis involved correlations between various measures of trophic level biomass. These included (1) planktivore biomass (or numbers) and zooplankton or *Daphnia* biomass, (2) planktivore biomass or numbers and the ratio of zooplankton or *Daphnia* biomass and chlorophyll *a*, (3) zooplankton or *Daphnia* biomass and measures of chlorophyll *a*, Secchi depth, or total pigments and the ratio of chlorophyll *a* with respect to total phosphorus, (4) concentrations of chlorophyll *a* and measures of Secchi depth with respect to total phosphorus, and (5) concentrations of chlorophyll *a* and winter or spring-turnover concentrations of total phosphorus.

We restricted our analyses to an investigation of simple correlations because we were interested only in the statistical significance of variables changing together, not in cause and effect. We avoided autocorrelation problems by eliminating correlations that involved organisms with similar turnover times and with turnover times that were long enough to influence mean annual data (i.e. piscivores and planktivores).

In order to allow comparisons with the large number of published chlorophyll–phosphorus regressions (Dillon and Rigler 1974; Schindler 1978; Stockner and Shortreed 1985; reviewed in McQueen et al. 1986), we derived correlation coefficients for mean annual concentrations of chlorophyll *a* with respect to total epilimnetic phosphorus. However, these correlation coefficients may be inflated because much of the phosphorus is found in the phytoplankton that produce chlorophyll *a* and so we have also calculated correlations for mean summer chlorophyll *a* concentrations with respect to total phosphorus independently measured during the preceding winter or during spring turnover. Throughout, we used ratios that have high correlation coefficients for numerators and denominators (Albrecht

TABLE 1. Oneida Lake mean annual data for May, June, July, and August. Estimates of piscivore biomass were derived from mark–recapture estimates and catches in trapnets at the Oneida Lake Hatchery for age 4 and older walleye in April. All chlorophyll *a*, zooplankton, Secchi depth transparency, and water chemistry data were collected on a weekly basis from May 1 to August 31 each year. The sample sizes (number of sampling weeks) are in square brackets. The number of samples is the same for total phosphorus, chlorophyll, total pigment, Secchi depth, total zooplankton, and daphnid biomass. Daphnid biomass comprises biomass estimates of *D. pulex* and *D. galeata*.

Year	Total phosphorus (μg/L)	Chlorophyll *a* (μg/L)	Total pigment (μg/L)	Secchi depth	Total zooplankton dry weight (μg/L)	Daphnid dry weight (μg/L)	Planktivore biomass (kg/ha)	Piscivore biomass (kg/ha)
1975	32.40 [18]	9.43	10.10	3.07	191.00	100.80	18.02 [13]	14.20
1976	24.39 [16]	7.90	8.50	3.00	227.80	169.80	6.56 [13]	19.80
1977	35.01 [18]	11.11	13.91	2.77	201.00	71.70	30.16 [15]	11.20
1978							7.85 [14]	18.20
1979	38.50 [16]	6.51	8.17	3.59	281.30	196.24	14.79 [13]	23.20
1980	37.01 [17]	10.60	11.14	2.90	273.50	186.60	25.51 [14]	13.70
1981	58.15 [17]	9.11	10.30	2.29	124.00	99.70	15.45 [13]	17.70
1982	45.29 [18]	8.06	8.79	2.27	229.90	115.40	24.29 [12]	15.20
1983	25.51 [18]	6.32	6.89	2.99	311.20	215.40	36.10 [15]	13.30
1984	26.16 [18]	8.28	8.48	2.57	315.00	220.00	7.80 [13]	22.10
1985	33.43 [17]	10.87	14.14	2.13	294.10	147.20	14.45 [12]	19.70
1986	42.94 [16]	10.53	11.20	2.26	382.00	263.40	3.33 [13]	19.50
1987	23.07 [17]	5.55	6.58	3.20	277.10	191.28	12.88 [13]	18.40
1988	31.35 [18]	5.91	6.66	2.85	342.70	186.80	6.78 [13]	18.00

TABLE 2. Gizzard shad index catches. Numbers of gizzard shad (per haul) captured in an 18-ft trawl.

Year	Number of trawls	Age 0+	Age >0+
1961	146	0	0
1962	126	0	0
1963	194	0	0
1964	150	0	0
1965	160	0	0
1966	160	0	0
1967	150	0	0
1968	130	0	0
1969	130	0	0
1970	140	0	0
1971	140	0	0
1972	110	1	1
1973	140	3	0
1974	130	2	0
1975	120	0	0
1976	120	6	0
1977	150	0	0
1978	140	1	0
1979	130	0	0
1980	140	0	0
1981	140	2	0
1982	120	0	0
1983	140	46	0
1984	130	79	0
1985	120	96	0
1986	130	21	0
1987	130	240	0
1988	130	19	0
1989	130	119	0

1978; Atchley and Anderson 1978; France 1990) and the ratios are correlated with independent variables (Jackson et al. 1990). Because $\log_{10}$ transformations did not improve the fit of the data and did not alter the statistical significance of any of the correlation coefficients, all values reported are for untransformed mean annual values. In all cases the one-tail level of significance for rejection of the null hypothesis is $\alpha(1) = 0.05$.

Results

Mean Annual Trends in the Oneida Lake Data Set (May through August) (Tables 1–3)

Walleye biomass in Oneida Lake is determined by rates of recruitment and angler harvest. Since 1975, walleye biomass has averaged 17.4 kg/ha, but April estimates have varied twofold over this period (range = 11–23 kg/ha (Table 1). Planktivore biomass has been more variable and is strongly influenced by the abundance of age 0 fish which varied 11-fold over a 4-yr period. The planktivore decline recorded since 1984 (Table 1) may be overly dramatic because it reflects the decreased numbers of yellow perch and white perch but does not include age 0 gizzard shad which are pelagic and have become more common in Oneida Lake since 1984 (Table 2). A gradually increasing trend in zooplankton biomass has occurred since the early 1980's and the biomass of the dominant daphnid species (*D. pulex* and *D. galeata*) has increased as well. May through August chlorophyll *a* concentrations averaged between 5.6 and 11.7 μg/L, while total phosphorus concentrations were more variable and ranged between 23 and 58 μg/L.

Correlations between planktivore biomass and the biomass of total zooplankton or the biomass of zooplankton per unit chlorophyll *a* were negative, but not significant (Table 3) at $\alpha(1) = 0.05$. Correlations between planktivore biomass and the biomass of *Daphnia* or the biomass of *Daphnia* per unit chlorophyll *a* were also negative, but not significant (Table 3). Correlations between zooplankton or *Daphnia* biomass and various measures of water clarity and chlorophyll *a* concentration were not significant (Table 3). The correlation between Secchi depth and mean epilimnetic phosphorus was negative and significant ($\alpha(1) = 0.05$) and the correlation between $\log_{10}$ chlorophyll *a* concentration and $\log_{10}$ total phosphorus was positive and significant. Finally the correlation between winter total phosphorus and chlorophyll *a* (Fig. 1) was also positive and significant.

TABLE 3. Oneida Lake (May–August) correlation coefficients. The coefficients noted with an asterisk are significant at $\alpha(1) = 0.05$ and the coefficient in square brackets is for $\log_{10}$:$\log_{10}$ data. Planktivores are measured as kg/ha. All other units are μg/L. The ratios of zooplankton and *Daphnia* per unit chlorophyll *a* and chlorophyll *a* per unit total phosphorus (TP) are defined under Materials and Methods. WTP is winter total phosphorus.

Planktivore biomass									
−0.32	Total zooplankton biomass								
−0.40	·	*Daphnia* biomass							
−0.21	·	·	Ratio of zooplankton per unit chlorophyll *a*						
−0.25	·	·	·	Ratio of *Daphnia* per unit chlorophyll *a*					
	−0.20	−0.32	·	·	Chlorophyll *a*				
	+0.01	+0.14	·	·	·	Secchi depth			
	+0.08	−0.01	·	·	·	·	Ratio of chlorophyll *a* per unit TP		
	−0.23	−0.42	·	·	·	·	·	Total pigments	
					+0.38	−0.48*			TP
					[+0.46]*				
					+0.56*				WTP

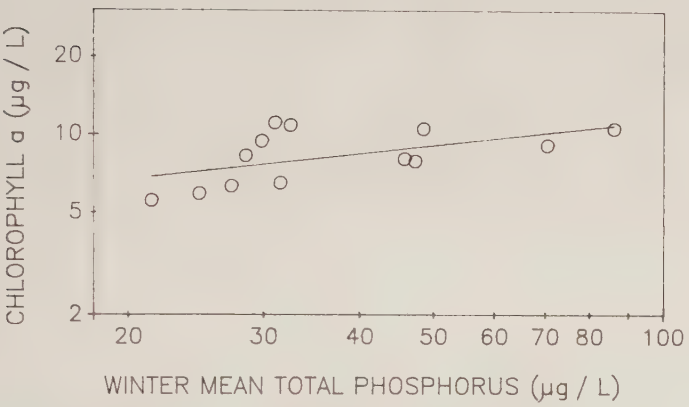

FIG. 1. Relationship of Oneida Lake mean summer epilimnetic chlorophyll *a* to winter mean total phosphorus.

Mean Annual Trends in the Oneida Lake Data Set. (July through September (Tables 4 and 5)

At Lake Oneida, 0+ planktivorous fish do not begin to feed heavily on zooplankton, and therefore potentially influence phytoplankton, until July and we assumed that this might explain the lack of top-down correlations observed in the May through August (inclusive) data set. We therefore repeated the entire analysis for a data set comprising samples collected during July to September (Table 4). The correlations for planktivore biomass versus biomasses of zooplankton and *Daphnia* were negative and not significant (Table 5). The biomasses of zooplankton and *Daphnia* versus various measures of phytoplankton abundance and water clarity had positive correlation coefficients rather than the negative correlation coefficients predicted by the various top-down hypotheses and none were significant (Table 5).

Mean Annual Trends in the Lake St. George Data Set (Tables 6 and 7)

At Lake St. George (Table 6), there was a partial winterkill in March 1982. The piscivores (northern pike, largemouth bass, and yellow perch >10 cm) and the planktivores (golden shiner, bluegill, young-of-the-year yellow perch, and yellow perch <10 cm) were sharply reduced. During the winterkill year, there was no detectable reproduction from any of the fish species, but during 1983 and 1984, the planktivore population rapidly increased. The piscivore population began to increase in 1985 as the winterkill survivors became sexually mature. As the piscivores increased the planktivores decreased. Zooplankton biomasses were high during 1982 when planktivores were low and low during 1984 when planktivores were high, but during other years the trends were not predictable. Chlorophyll *a* concentrations were high during 1980–83 and lower during 1984–88. Total epilimnetic phosphorus concentrations were highest during 1980–83 and lower during 1984–88.

The correlations for planktivore biomass versus biomasses of zooplankton and *Daphnia* and *Ceriodaphnia* were negative and not significant (Table 7). The correlations for biomasses of zooplankton and *Daphnia* versus various measures of phytoplankton abundance and water clarity were not significant (Table 7). The correlation between Secchi depth and mean epilimnetic phosphorus was negative and significant ($\alpha(1) = 0.05$) and the correlation between $\log_{10}$ chlorophyll *a* concentration and $\log_{10}$ total phosphorus was positive and significant. These results are in agreement with many other studies. The correlation between spring-turnover total phosphorus and mean summer chlorophyll *a* (Fig. 2) was also positive and significant (Table 7).

Finally, when the Oneida Lake and Lake St. George mean summer chlorophyll *a* and epilimnetic total phosphorus data were combined (Fig. 3), the resulting regression line ($r^2 = 0.43$) almost exactly matched the regression of Janus and Vollenweider (1981) which is based on data from 140 lakes.

Time Trends

Because the Lake St. George community suffered a partial winterkill during the winter of 1981–82 (described in McQueen et al. 1989), we wanted to test the hypothesis that the cascading effects due to this strong "biomanipulation" would be more obvious shortly after the event than later when the system returned to premanipulation equilibrium. We investigated this hypothesis by sequentially removing years from the data set and observing the effects on correlation coefficients and associated probability values. Because we were interested only in changes over time and not in between-trophic-level comparisons within the food web, we chose to simplify the analysis as much as possible by including only the most obvious interactions (Table 8). We also included coefficients for planktivores as a function of piscivores even though we recognized that fish live for more than 1 yr, and therefore, coefficients based on mean annual data might be inflated by nonindependence.

This analysis showed several trends (Table 8). The most significant top-down between-fish effects were observed 5 yr after

TABLE 4. Oneida Lake mean annual data for July, August, and September. Estimates of piscivore biomass were derived from mark–recapture estimates and catches in trapnets at the Oneida Lake Hatchery for age 4 and older walleye in April. All chlorophyll *a*, zooplankton, Secchi depth transparency, and water chemistry data were collected on a weekly basis from July 1 to September 30 each year. The sample sizes (number of sampling weeks) are in square brackets. The number of samples is the same for total phosphorus, chlorophyll, total pigment, Secchi depth, total zooplankton, and daphnid biomass. Daphnid biomass comprises biomass estimates of *D. pulex* and *D. galeata*.

Year	Total phosphorus (μg/L)	Chlorophyll *a* (μg/L)	Total pigment (μg/L)	Secchi depth	Total zooplankton dry weight (μg/L)	Daphnid dry weight (μg/L)	Planktivore biomass (kg/ha)	Piscivore biomass (kg/ha)
1975	60.87 [13]	13.57	14.51	1.88	129.35	5.67	18.02 [13]	14.20
1976	35.29 [11]	15.14	15.27	2.34	244.41	193.35	6.56 [13]	19.80
1977	37.19 [12]	15.27	19.30	1.83	120.51	59.93	30.16 [15]	11.20
1978							7.85 [14]	18.20
1979	63.43 [13]	21.16	28.53	1.25	271.70	149.18	14.79 [13]	23.20
1980	48.95 [13]	17.40	17.52	2.22	227.85	140.51	25.51 [14]	13.70
1981	98.52 [13]	14.78	15.88	1.79	109.11	6.43	15.45 [13]	17.70
1982	59.22 [13]	11.96	12.75	1.62	229.78	83.71	24.29 [12]	15.20
1983	43.65 [13]	10.17	11.20	2.25	250.49	163.52	36.10 [15]	13.30
1984	34.55 [13]	13.22	13.82	1.71	234.86	137.79	7.80 [13]	22.10
1985	37.47 [14]	14.55	18.87	1.63	224.26	74.22	14.45 [12]	19.70
1986	70.64 [13]	14.11	14.23	2.12	341.39	256.90	3.33 [13]	19.50
1987	29.83 [12]	8.05	9.49	2.18	120.51	55.36	12.88 [13]	18.40
1988	43.26 [13]	8.57	9.14	2.73	260.68	129.15	6.78 [13]	18.00

TABLE 5. Oneida Lake (July–September) correlation coefficients. Planktivores are measured as kg/ha. All other units are μg/L. The ratios of zooplankton and *Daphnia* per unit chlorophyll *a* and chlorophyll *a* per unit total phosphorus (TP) are defined under Materials and Methods.

Planktivore biomass									
−0.32	Total zooplankton biomass								
−0.30	·	*Daphnia* biomass							
−0.24	·	·	Ratio of zooplankton per unit chlorophyll *a*						
−0.25	·	·	·	Ratio of *Daphnia* per unit chlorophyll *a*					
	+0.15	+0.13	·	·	Chlorophyll *a*				
	+0.13	+0.29	·	·	·	Secchi depth			
	+0.02	+0.18	·	·	·	·	Ratio of chlorophyll *a* per unit TP		
	+0.08	+0.03	·	·	·	·	·	·	Total pigments
					+0.32	−0.29		+0.22	TP

TABLE 6. Lake St. George mean annual data. The estimates for fish come from mark–recapture assessments conducted in the fall of each year. The other values come from weekly or biweekly samples collected during May, June, July, and August of each year. The data sources which give detailed methods and estimates of variability are McQueen et al. (1986, 1989, 1990) and Johannes et al. (1989). The sample sizes (number of sampling weeks) are in square brackets. The number of samples is the same for daphnids and zooplankton. Fish were assessed by mark and capture.

Year	Total epilimnetic phosphorus (μg/L)	Chlorophyll *a* (μg/L)	Secchi depth	Total zooplankton wet weight (μg/L)	Daphnid wet weight (μg/L)	Planktivore number/ha	Piscivore number/ha
1980	33.3 [18]	8.5 [17]	1.7 [17]	—	—	8562	980
1981	32.5 [8]	7.0 [9]	1.5 [13]	2361 [8]	754	9715	1782
1982	32.0 [13]	9.8 [12]	1.8 [16]	4596 [12]	2104	2488	500
1983	33.5 [5]	5.2 [6]	1.5 [13]	4541 [17]	1909	12897	660
1984	25.7 [14]	3.3 [16]	1.4 [19]	2472 [15]	239	20821	523
1985	17.5 [5]	3.7 [11]	2.8 [18]	4773 [17]	1727	13406	680
1986	24.2 [8]	5.4 [21]	2.4 [17]	2990 [16]	827	7746	1060
1987	24.2 [16]	5.1 [16]	1.5 [16]	2800 [16]	912	5794	1362
1988	23.5 [14]	3.4 [14]	2.4 [14]	2617 [13]	707	3666	1004
1989	29.4 [20]	5.8 [20]	—	—	—	3649	503

the winterkill when the planktivore population had increased 10-fold and then decreased as piscivores began to recruit back into the system. As years were added to the data set and the system approached prewinterkill levels, the correlations weakened. The top-down correlations between planktivores and zooplankton were most obvious 2–3 yr after the winterkill as the planktivore community increased and zooplankton biomass and individual size decreased, but the relationships between zooplankton or *Daphnia* and chlorophyll *a* were never significant. After the addition of the 1986 data, the correlations between

TABLE 7. Lake St. George summer correlation coefficients. The coefficients noted with an asterisk are significant at $\alpha(1) = 0.05$ and the coefficient in square brackets is for $\log_{10}$:$\log_{10}$ data. Planktivores are measured as numbers/ha. All other units are μg/L. The ratios of zooplankton and *Daphnia* per unit chlorophyll *a* and chlorophyll *a* per unit total phosphorus (TP) are defined under Materials and Methods. STP is spring total phosphorus.

Planktivore numbers								
−0.05	Total zooplankton biomass							
−0.28	·	*Daphnia* biomass						
+0.43	·	·	Ratio of zooplankton per unit chlorophyll *a*					
+0.03	·	·	·	Ratio of *Daphnia* per unit chlorophyll *a*				
	+0.29	+0.51	·	·	Chlorophyll *a*			
	+0.29	+0.51	·	·	·	Secchi depth		
	+0.38	+0.55	·	·	·	·	Ratio of chlorophyll *a* per unit TP	
					+0.72*	−0.71*	TP	
					[+0.71]*			
					+0.86*			STP

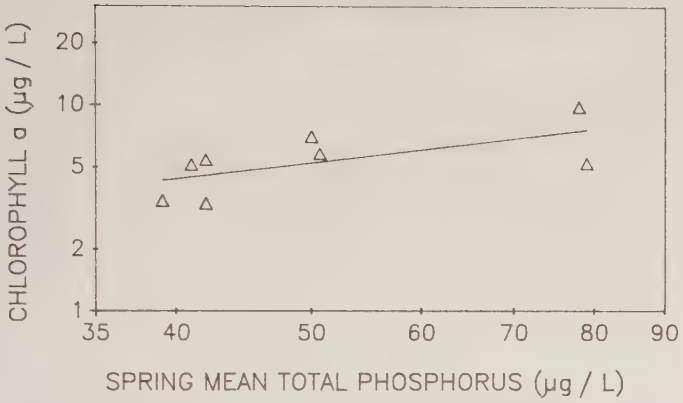

FIG. 2. Relationship of Lake St. George mean summer epilimnetic chlorophyll *a* to April turnover-time mean total phosphorus.

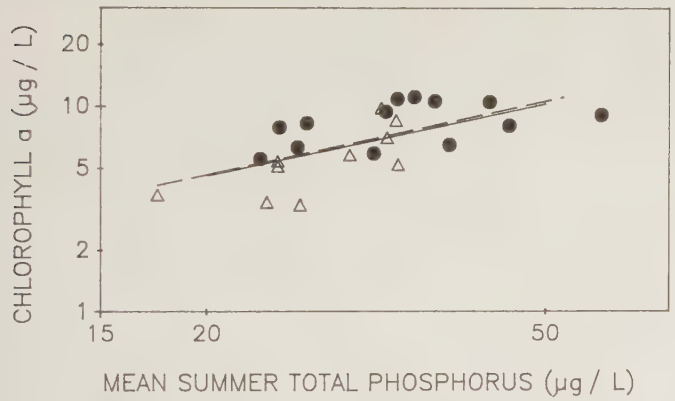

FIG. 3. Lake St. George (open triangles) and Oneida Lake (filled circles) mean summer epilimnetic chlorophyll *a* plotted with respect to mean summer epilimnetic total phosphorus. The broken line is the line of best fit through the data. The solid line is that of Janus and Vollenweider (1981) based on data from 140 lakes.

chlorophyll *a* and total phosphorus were all positive and the significance increased with the addition of each succeeding year.

Between-Lake Comparisons

With one exception, the summer relationships for Lake St. George were similar to those found at Lake Oneida, the exception being the relationships between zooplankton (or daphnids) and measures of phytoplankton abundance (chlorophyll *a*, ratio of chlorophyll *a* per unit total phosphorus). For the Oneida Lake data set, these relationships are negative (but not significant) (Table 3). For the Lake St. George data set, they were positive (also not significant) (Table 7). At Oneida Lake, zooplankton biomass is generally dominated by daphnids and when the large-bodied *D. pulex* is abundant, algal density declines and Secchi depth transparency increases (Mills et al. 1987). At Lake St. George, fewer of the zooplankton comprise *Daphnia* and large-bodied *D. pulex* are not present. Occasionally, however, large-bodied (length >1 mm) *D. galeata* have dominated the zooplankton assemblage. For example, during 1985 (Table 6), large populations of large *D. galeata* were associated with unusually low concentrations of chlorophyll *a* and with exceptionally high Secchi depth transparency (Table 6). Additionally, when we correlated mean monthly counts (numbers per litre) for *D. galeata* >1 mm with mean monthly chlorophyll *a* concentrations (Fig. 4), the relationship was negative like the relationships found at Oneida Lake, but it was also not significant.

Discussion

Mean Annual Trends

For both lakes, the long-term mean annual trends for relationships between planktivore abundance and various measures of zooplankton abundance were usually negative and were only significant for Lake St. George 2–3 yr directly after the winterkill when the fish communities were still influenced by strong postmanipulation effects. The mean annual trends for correlations between zooplankton (or *Daphnia*) and various measures of phytoplankton abundance were never significant, had both negative and positive slopes, and were not influenced by time trends following biomanipulations. The mean annual trends for chlorophyll *a* and various measures of total phosphorus availability (mean summer epilimnetic, winter, spring turnover) showed consistent positive correlations that were frequently statistically significant and became stronger as the data set increased. Based on these trends, we conclude that for Oneida Lake and Lake St. George, the cascading effects of changes at the top of the food web (piscivores) are almost never significant over the long term and become weaker and more unpredictable as they are transmitted down through the planktivore trophic level to the zooplankton and phytoplankton.

These trends based on long-term data are not in agreement with the results of several short-term studies which have shown that there are significant negative associations over days or weeks between zooplankton and phytoplankton biomass

TABLE 8. Lake St. George correlation coefficients for six trophic level interactions. The coefficients were calculated for data sets ranging from 1980 through 1989. Concentrations of fish are measured as numbers/ha and concentrations of the other variables as μg/L. Significance at $\alpha(1) = 0.05$ is noted with an asterisk.

Correlated variables	Data sets comprising data from 1980 to the following years:						
	1983	1984	1985	1986	1987	1988	1989
Piscivores, planktivores	−0.51	−0.66	−0.65	−0.62	−0.68*	−0.58*	−0.33
Planktivores, zooplankton		−0.23	−0.56	−0.43	−0.32	−0.19	−0.05
Planktivores, *Daphnia*		−0.34	−0.74	−0.68	−0.55	−0.44	−0.28
Zooplankton, chlorophyll *a*		+0.14	+0.48	+0.18	+0.18	+0.20	+0.29
Daphnia, chlorophyll *a*		+0.25	+0.66	+0.45	+0.45	+0.46	+0.51
Chlorophyll *a*, total phosphorus	−0.34	−0.68	+0.65	+0.68*	+0.68*	+0.69*	+0.72*

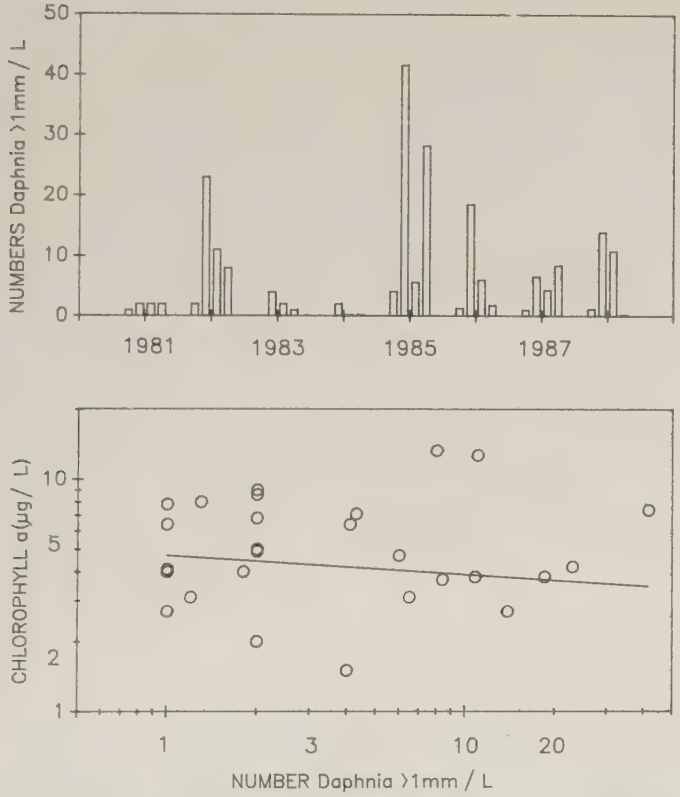

FIG. 4. (Top panel) Lake St. George monthly mean counts for *Daphnia* >1 mm in length; (bottom panel) relationship between numbers of *Daphnia* >1 mm and chlorophyll *a* concentration.

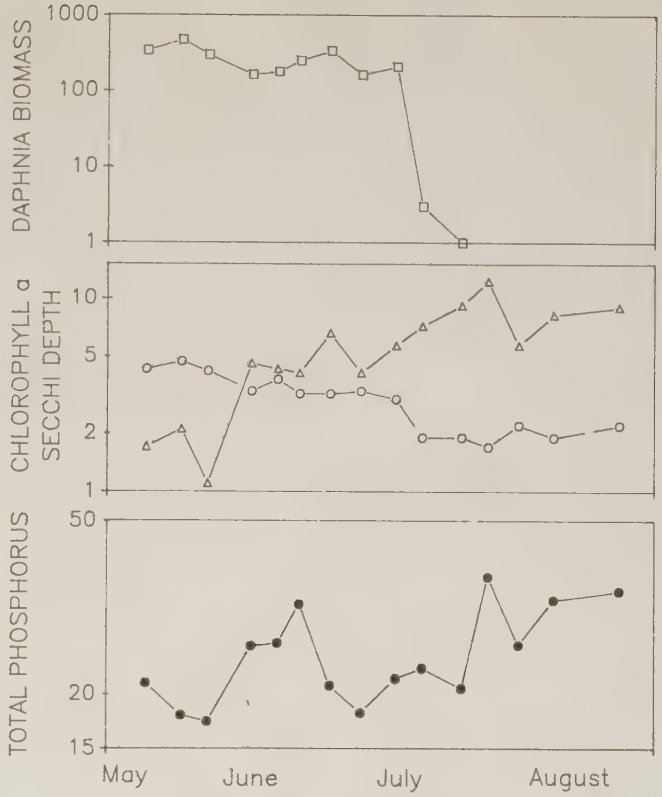

FIG. 5. Oneida Lake (1987) total phosphorus (μg/L), Secchi depth (m) (open circles), chlorophyll *a* (μg/L) (open triangles), and *D. pulex* (μg/L dry weight).

(Carpenter et al. 1987). Also, "clear water phases" lasting a few weeks have been reported by several authors and in at least one instance (Lampert et al. 1986) have been convincingly attributed to zooplankton grazing. At both Lake St. George (McQueen et al. 1989) and Oneida Lake, these short-term events have been documented. For example, at Oneida Lake, after 1984, piscivore densities remained at relatively high levels and densities of yellow perch and white perch declined (Table 1) significantly. During this period, gizzard shad became more abundant (Table 3) and were especially numerous during 1987. As the summer of 1987 progressed, this short-term increase in gizzard shad density was associated with increased concentrations of chlorophyll *a*, decreased Secchi depths, and decreased populations of *D. pulex* (Fig. 5). Because total phosphorus also increased, the observed changes in chlorophyll *a* and Secchi depth could not be entirely attributed to zooplankton grazing, but there was a clear, short-term, empirical top-down relationship between changes in planktivore biomass and changes in phytoplankton.

These short-term events contribute significantly to our understanding of potential interactions between pelagic predators and their prey, but do they accurately predict food web responses to long-term biomanipulations of fish communities? We think not. We base this assertion on the observation that changes in annually reproducing temperate fish populations are best described by annual data. Because biomanipulation theory makes predictions about the responses that the plankton community has to these annual changes, we suggest that mean annual plankton values are most appropriate. They allow assessments of before and after treatment interannual effects and they allow between-lake comparisons.

Our analysis of these mean annual responses suggests that they are unpredictable. For example, at Oneida Lake, the highest *Daphnia* and total zooplankton biomasses were recorded

during 1986 and were associated with the second highest pigment level (Table 1), the third highest chlorophyll *a* level (Table 1), and the second lowest Secchi depth (Table 1). Conversely very low concentrations of chlorophyll *a* and pigments were observed during 1979, 1983, and 1987 (Table 1) and were associated with intermediate concentrations of *Daphnia*). At Lake St. George (Table 6), 1982 and 1985 were the years of maximum zooplankton and daphnid biomass; but in 1985, chlorophyll *a* levels were near the lowest recorded, and in 1982 they were the highest recorded. These mean annual data do not help us to understand the mix of events that created the patterns that were observed in the two lakes, but they do accurately represent the major trends over the spring to fall growth period. They also lead to the conclusion that at Oneida Lake and Lake St. George, top-down responses to long-term changes in fish community structure do not account for a significant portion of observed interannual variability.

Sustainability

At both lakes, the fish communities were strongly influenced by a number of external forces (i.e. angler harvest and winterkill) and by highly variable recruitment. The result was that numbers and biomasses showed substantial variation. For Oneida Lake, piscivores varied by approximately 100% and planktivores by more than 300% (Table 1). At Lake St. George, piscivores varied by approximately 70% and planktivores by 500% (Table 6). As noted earlier, these changes were insufficient to cause sustained interannual changes in phytoplankton biomass. However, in other lakes, manipulations of fish biomass have resulted in significant changes in zooplankton and phytoplankton biomass and species composition. This is especially true for lakes where all of the fish have been removed by poison (Reinertsen et al. 1990) or by draining (Meijer et al. 1990).

The differences between these examples and the events that we have described for Oneida Lake and Lake St. George are (1) that the "successful" biomanipulations were massive and (2) that they completely restructured the aquatic community. At Oneida Lake and Lake St. George, changes in the piscivore community were substantial, but the basic structure of the food web was not changed and compensatory changes within the community effectively damped the top-down cascades before they reached the phytoplankton. Furthermore, the inherent resilience of both communities quickly corrected changes at the top of the food web. For example, Mills et al. (1987) have shown that intensity of walleye cannibalism is inversely related to the survival of 0+ walleye recruits and that this results in well-described year-class effects which make it virtually impossible to sustain high populations of piscivores.

Some of these processes are well illustrated by Carpenter (1989) who used a model community to analyze trophic responses to changes in piscivore biomass and demonstrated that piscivore concentrations would have to change by at least an order of magnitude before statistically reliable changes in phytoplankton could be observed. This suggests that if the changes in the fish communities at Oneida Lake and Lake St. George had been much greater and that if the new fish community was maintained in its new configuration by substantial and constant intervention (Benndorf 1990), changes may have cascaded down to the phytoplankton.

However, the question of interest is whether a freshwater community can sustain the massive changes in fish community structure that are required to cause predictable changes in phytoplankton abundance. This question becomes especially important if the high biomasses of piscivores which are needed to induce predictable changes in the lower trophic levels have compensatory impacts on piscivore production. Examples of sustained high levels of piscivore stocking have now been documented in Lakes Ontario and Michigan where salmonid stocking has resulted in substantial changes in alewife (*Alosa pseudoharengus*) (Scavia et al. 1986), and recent evidence from Lake Michigan shows that growth, survivorship, and conversion rates (Stewart and Ibarra 1991) among the stocked fish are falling. This suggests that the systems may have reached their capacities to absorb salmonid stocking, yet even at these high levels of salmonid biomass, the effects on phytoplankton are not strong enough to constitute clear evidence for a top-down cascade (Lehman 1988; Lehman and Sandgren 1990; Sandgren and Lehman 1990). Perhaps the inescapable conclusion is that the enormous changes in the piscivore community that are required to cause changes in phytoplankton abundance cannot be supported by the pelagic community.

Acknowledgements

The Oneida Lake research was supported by grants from Cornell University and the New York State Department of Environmental Conservation. This is contribution 114 of the Cornell Biological Field Station. The Lake St. George work was supported by the Natural Sciences and Engineering Research Council of Canada, the Ontario Ministry of Natural Resources, and Environment Canada.

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Planktonic Sea Scallop Larvae (*Placopecten magellanicus*) in the Georges Bank Region: Broadscale Distribution in Relation to Physical Oceanography

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Tremblay, M. J., and M. Sinclair. 1992. Planktonic sea scallop larvae (*Placopecten magellanicus*) in the Georges Bank region: broadscale distribution in relation to physical oceanography. Can. J. Fish. Aquat. Sci. 49: 1597–1615.

During autumn surveys for sea scallop larvae (*Placopecten magellanicus*) from 1985 to 1987, the mean abundance of sea scallop larvae on the northeast part of Georges Bank (1201–20080·m^{-2}) was much greater than on the southern Scotian Shelf (5–240·m^{-2}). Few larvae were collected between these two areas, and exchange between Georges Bank and the Scotian Shelf appears very limited. Transects across the northern flank in 1988 revealed peaks in larval abundance when on-bank temperature stratification was high. Relative larval abundance on the transects was positively related to the speed of the along-front current, suggesting physical convergence of larvae. The on-bank retention of scallop larvae on Georges Bank appears to be due to physical processes alone, since scallop larvae undertake only limited diel vertical migration. Larval exhange among adult scallop aggregations on Georges Bank (the northeast peak, the southeast part, and the South Channel) is probable, but evidence from this study is limited. The autumn production of late-stage larvae on the northern flank and northeast peak of Georges Bank is estimated to range from 120 to 1500·m^{-2}, which is 10–100 times greater than the density of scallops aged 1–2 yr.

Lors des études effectuées sur les larves de pétoncle géant (*Placopecten magellanicus*) entre 1985 et 1987, l'abondance moyenne des larves de pétoncle géant dans la région nord-est du Banc Georges (1 201–20 080·m^{-2}) a été bien supérieure à celle dans la Plate-forme Scotian plus méridionale (5–240·m^{-2}). Entre ces deux régions, peu de larves ont été récoltées et l'échange entre le Banc Georges et la Plate-forme Scotian semble très limité. Des transects à travers le flanc septentrional en 1988 ont révélé des pics d'abondance des larves lorsque la stratification thermique était élevée sur les bancs. On a mis en évidence une relation positive entre l'abondance relative des larves sur les transects et la vitesse du courant, ce qui pourrait indiquer une convergence physique des larves. La rétention des larves de pétoncle géant sur le Bank Georges semble être attribuable à un processus physique seulement, car la migration verticale nycthémérale des larves de pétoncle est limitée. Des échanges de larves ont probablement lieu entre les regroupements de pétoncles adultes sur le Banc Georges (le nez au nord-est, la partie sud-est et le chenal sud), mais cette étude n'en a fourni que peu de preuves. On estime que la production automnale de larves à un stade de développement avancé sur le flanc nord et le nez au nord-est du Banc Georges varie entre 120 et 1 500·m^{-2}, ce qui est de 10 à 100 fois supérieur à la densité des pétoncles âgés de 1 à 2 ans.

Received August 13, 1991
Accepted February 7, 1992
(JB180)

Reçu le 13 août 1991
Accepté le 7 février 1992

The extent of mixing among sea scallop (*Placopecten magellanicus*) aggregations on Georges Bank and adjacent waters (Fig. 1) is not well understood (Sinclair et al. 1985). If exchange among aggregations occurs, it most likely takes place during the planktonic larval phase because movement by scallops on the bottom is thought to be minimal and undirected (Baird 1954; Posgay 1981; Melvin et al. 1985). It has long been assumed that planktotrophic larvae function to increase the range of the parent populations (Thorson 1946), but larval dispersal may be less extensive than expected (Burton and Feldman 1982; Hedgecock 1986; Sinclair 1988). The larval phase may also be important to recruitment, since high retention or survival of larvae has been linked to successful recruitment to fishable sizes (Dickie 1955; Caddy 1979, 1989).

Knowledge of the adult distribution and physical oceanography is important for interpreting the extent to which planktonic larvae are dispersed. The generalized distribution of sea scallops in the Georges Bank region is depicted in Fig. 1. Landings by the fishery (Table 1) are a coarse index of the relative abundance of scallops in these areas, since fishing effort is high (Sinclair et al. 1985). Although differences in fishing effort and management measures among areas limit the value of landings for the detection of small annual fluctuations in abundance, the larger catch on Georges Bank from 1984 to 1988 shows that scallops are much more abundant there than in the surrounding area. Scallops also tend to be more concentrated on Georges Bank, a trend reflected by catch per unit effort during stock surveys (Table 1). Scallop fishing on most of the Scotian Shelf

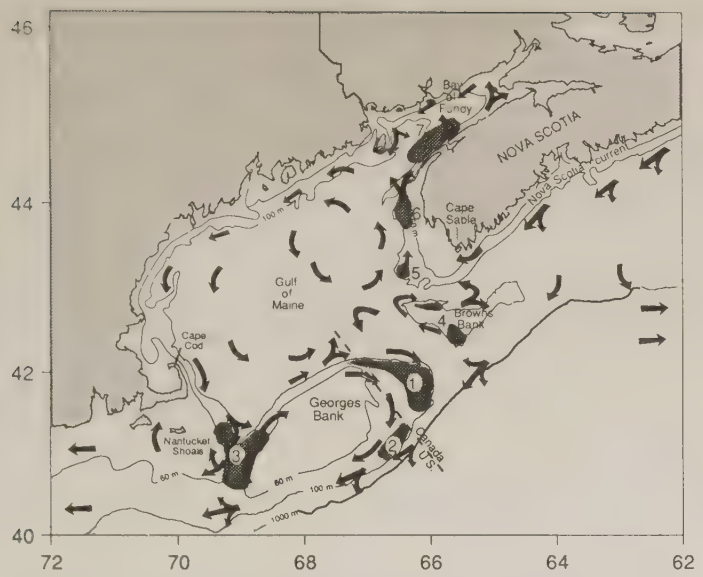

FIG. 1. Major fishing aggregations for sea scallops (modified from Sinclair et al. 1985 and Robert et al. 1989b), and residual circulation in the Georges Bank and Scotian Shelf areas. Residual circulation from unpublished figure by R. Trites, Bedford Institute of Oceanography, and Brooks (1985). Numbered fishing areas are as follows: 1, Northern flank and northeast peak; 2, southeast part; 3, South Channel; 4, Browns Bank; 5, German Bank; 6, Lurcher Shoals; 7, Bay of Fundy. Recent landings for these areas are given in Table 1.

can be characterized as sporadic, highly localized, and opportunistic (Jamieson et al. 1981; Robert et al. 1989b).

On Georges Bank, three main fishing areas were recognized by Serchuk et al. (1979) and Sinclair et al. (1985): the northeast peak, the southeast part, and the South Channel (aggregations 1, 2, and 3 on Fig. 1). The northeast peak is generally the most productive area in terms of the fishery, contributing ~70% of the total catch (Serchuk et al. 1979; Serchuk and Wigley 1988) (Table 1). Annual Canadian stock surveys of sea scallops on the northeast peak show that animals between the ages of 3 and 11 yr are most concentrated in a crescent-shaped area at depths of 60–100 m (Fig. 2). Sea scallops are in low abundance in the well-mixed part (less than ~50 m) of the bank on both Canadian and U.S. sides (Serchuk and Wigley 1986).

The major aggregations of sea scallops in the Gulf of Maine area tend to occur in the vicinity of frontal zones between vertically well-mixed and stratified waters (Fig. 2) (Sinclair et al. 1985; Horne et al. 1989). The residual circulation of these areas (Fig. 1, 3) often includes semiclosed gyres (Greenberg 1983; Loder et al. 1988). Thermohaline gradients, tides, wind, and Gulf Stream rings will all contribute to variability in the residual or mean circulation.

The Georges Bank gyre is "leaky": drifters do exit from the bank, in an apparently unpredictable manner (Colton and Temple 1961; Bumpus 1976; Butman et al. 1982; Loder et al. 1988). However, Georges Bank is retentive compared with other submarine banks in the region (Loder et al. 1988). Consideration of residence time (a measure of cross-isobath exchange which gives the average time that particles will stay over the bank) and recirculation time (time for particles to complete a circuit of the bank) indicates that larvae which remain planktonic for more than a few weeks, such as the larvae of sea scallops, have a greater potential to recirculate on Georges Bank than on many other banks.

The front between the shallow, tidally well-mixed waters and deeper, more stratified waters of Georges Bank (Fig. 2) has several well-defined features (Loder et al. 1992). These include a sea-surface temperature gradient during summer and a subsurface temperature front intersecting the bottom at depths of 69–175 m (Yentsch and Garfield 1981; Loder and Greenberg 1986; Flagg 1987; Loder et al. 1992). Horne et al. (1989) emphasized that the tidal front should be thought of as occurring as a zone 10–40 km wide, rather than a precise location (Fig. 2a). The extent of physical exchange across the front is not well understood but is under active study (Loder and Platt 1985; Horne et al. 1989; Loder et al. 1992).

Many Georges Bank plankton surveys indicate that the exchange of larvae or zooplankton with the Gulf of Maine and Scotian Shelf is limited (haddock larvae: Smith and Morse 1985; Hurley and Campana 1989; chaetognaths: Clarke et al. 1943; Lough and Trites 1989; herring larvae: Boyar et al. 1973; Schnack and Stobo 1973; Bumpus 1976; Iles and Sinclair 1982; cod larvae: Hurley and Campana 1989; copepods: Davis 1984). Several of these studies also show temporal shifts in the distribution of plankters in the direction of the Georges Bank gyre and an increase in the percentage of older plankters downstream from spawning locations.

In contrast with the above, some studies suggest that extensive losses of larvae from Georges Bank do occur or that the

TABLE 1. Recent landings (tonnes of meats) for aggregations of sea scallops on Georges Bank, parts of the Scotian Shelf, and the Bay of Fundy (see Fig. 1). Data for Georges Bank include Canadian and U.S. catches. Bay of Fundy season runs over 2 yr and the figures include landings from the previous year. In parentheses are catch rate data for some areas. Catch rate is number of scallops (age 3 yr and older) per standard tow caught with a lined offshore scallop dredge in high-density strata during Canadian stock surveys. Landings data are from Robert et al. (1989a, 1989b), Serchuk and Wigley (1988), and F. Serchuk (National Marine Fisheries Service, Woods Hole, MA, pers. comm.). Catch rate data are from Mohn et al. (1989), Robert et al. (1989b), and Robert and Butler (1991).

	Georges Bank						
Year	Northeast part	Southeast part	South Channel	Total	Browns Bank	German/ Lurcher	Bay of Fundy
1984	2692 (62)	691	1633	5016	30 (60)	240	717
1985	4804 (282)	403	1554	6761	16 (2)	37 (27)	601
1986	5783 (393)	654	2744	9181	5 (2)	36	425
1987	9009 (207)	265	2404	11678	0	1	130
1988	6460 (176)	835	3124	10419	5	1	1226
Mean	5750	570	2292	8611	11	63	620

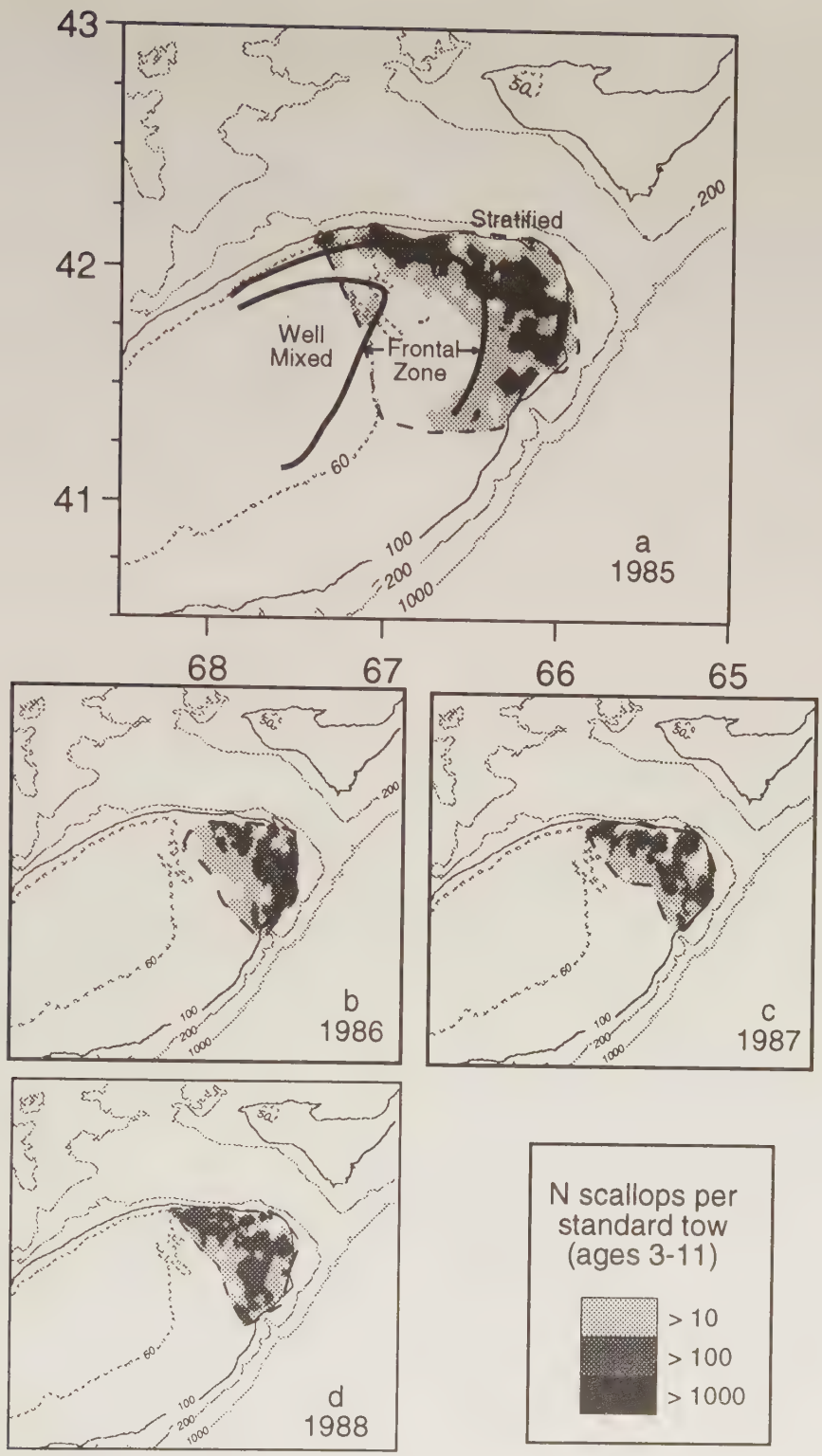

FIG. 2. Distribution of sea scallops (ages 3–11) on Georges Bank from Canadian research surveys from 1985 to 1988. (a) Number of tows = 200; (b–d) number of tows = 150. Tow lengths are standardized to 800 m (survey methods are described in Robert and Jamieson 1986). Heavy broken line in each panel indicates survey boundary. Heavy solid line in Fig. 2a shows position of the frontal zone in July 1985 using the criterion of 0- to 50-m density difference of 0.2–1.5 sigma-t units (from Horne et al. 1989).

residual circulation does not fully explain the pattern of larval distribution. From plankton surveys, Walford (1938) concluded that in 1931, haddock eggs and larvae were retained on Georges Bank but that in 1932, extensive losses occurred to the north and south of the bank. Colton and Temple (1961) inferred from drifter studies and the distribution of spawning haddock and herring that large losses of eggs and larvae often occur off the southern side of Georges Bank, but they were unable to substantiate these losses. Later drifter studies (Colton and Anderson 1983) again suggested extensive losses of larvae from

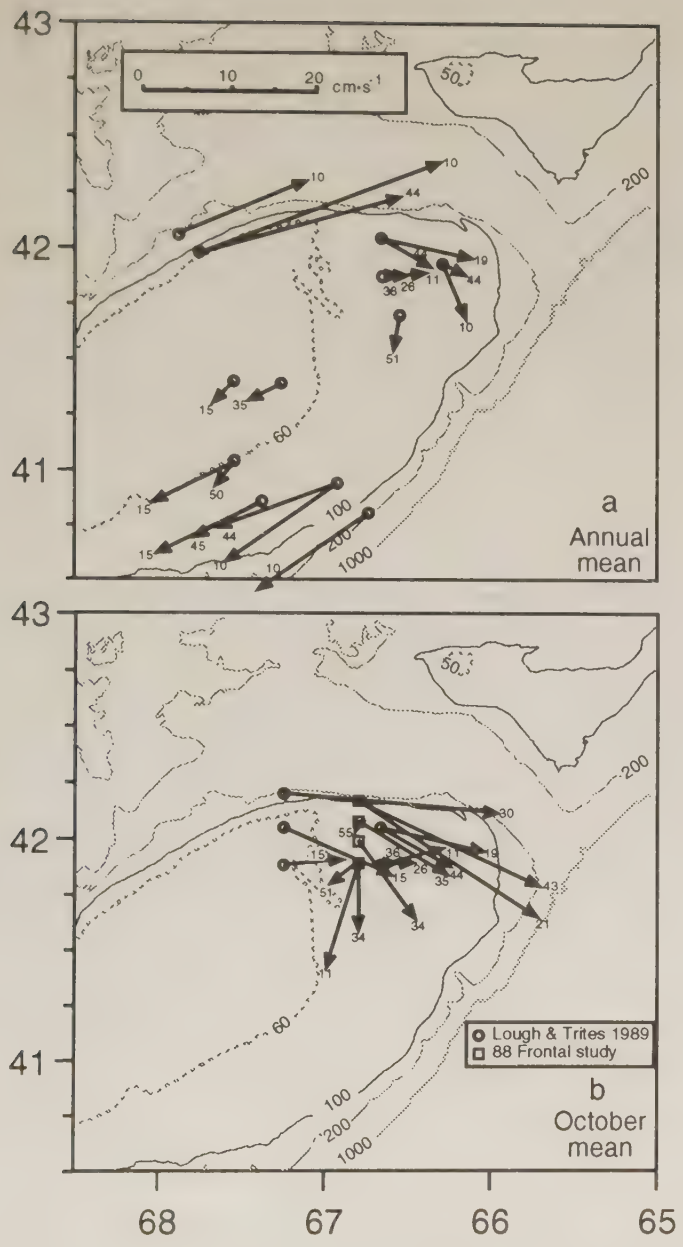

FIG. 3. Current speed and direction on Georges Bank from moored current meters. Vectors represent the mean of measurements of at least 2 wk. Scale reflects the distance a particle would move in 5 d if it travelled at the speed and direction measured at the mooring. Depths of measurements (at arrow tips) range over the depths where sea scallop larvae are most concentrated on Georges Bank (10–50 m). (a) Mean annual residual circulation for various seasons and periods (41–1113 d) from 1975 to 1979 (from Butman et al. 1982); (b) October measurements from 1988 Georges Bank Frontal Study (October 1–14; Loder and Pettipas 1991) and Lough and Trites (1989; October 17–30, 1978).

the south side of the bank, but no relationships were found between drifter direction and larval abundance or mortality. Lobster larvae have also been hypothesized to be exported from Georges Bank. Based on surface currents, water temperature, landings trends, and the distribution of large females and late-stage larvae, Harding et al. (1983) reasoned that lobster larvae from the northeast peak of Georges Bank seeded the large lobster stocks along the southern coast of Nova Scotia and the inner Gulf of Maine.

The circulation associated with tidal fronts may be important to the distribution of herring larvae (Iles and Sinclair 1982; Sinclair and Iles 1985), scallop larvae (Sinclair et al. 1985), and a host of other planktonic animals (Pingree et al. 1974; Le Fèvre 1986; Taggart et al. 1989; Perry et al. 1990). Frontal circulation may control the distribution of passive larvae, or plankton may have behavior which "uses" the circulation to effect retention or enhance dispersal. The potential for an active component in the dispersal of planktonic larvae is recognized (Rothlisberg 1982; Norcross and Shaw 1984; Sulkin 1984; Mann 1986; Stancyk and Feller 1986). On Georges Bank, vertical migrations by chaetognaths can retard horizontal transport (Lough and Trites 1989), and a behavioral mechanism may be involved in the retention of the larvae of Georges Bank herring (Iles and Sinclair 1982), cod, and haddock (Lough and Bolz 1989).

TABLE 2. Dates of plankton surveys during which sea scallop larvae were collected. Ship was the RV *Lady Hammond* except for the September 1987 survey when samples were obtained from the RV *Delaware II*. In 1986 and 1987, parts of the survey grid were sampled twice (a and b). The 1988 cruises were part of the Georges Bank Frontal Study and were restricted to the northern flank and northeast peak.

Year	Date
1985	Oct. 8–11
1986	(a) Sept. 30 – Oct. 8
	(b) Oct. 8–13
1987	Sept. 5–7
	(a) Sept. 29 – Oct. 8
	(b) Oct. 12–19
1988	Aug. 27 – Sept. 1
	Oct. 5–19

This paper has two objectives. The first is to relate the horizontal distribution and abundance of sea scallop larvae in the Georges Bank region to the physical oceanography and infer the extent of larval dispersal among fished aggregations. In particular, we examine the questions of whether scallop larvae are lost from Georges Bank to the surrounding area, whether larvae on Georges Bank are transported in the direction of the residual circulation, and whether the frontal zone imposes any structure on the spatial distribution of scallop larvae. These questions are examined through the analysis of data from (i) broadscale plankton surveys over the northern part of Georges Bank and southern part of the Scotian Shelf and (ii) plankton transects across the frontal zone on the northern flank.

The second objective of this paper is to compare the mean abundance of late-stage larvae on Georges Bank with recent abundance estimates for juvenile scallops and draw some implications for the recruitment process in sea scallops.

Methods

Surveys

The samples used to describe the broadscale distribution of sea scallop larvae were obtained during six cruises over four years (Table 2). Most samples were collected during the October cruises of 1985, 1986, and 1987, with additional samples obtained by opportunity on cruises undertaken in early September 1987 and in late August 1988. These latter surveys allow a better depiction of the temporal distribution of larvae on Georges Bank.

The survey design was systematic and stations were either on transects (1985, 1988) or a grid (1986, 1987). In October

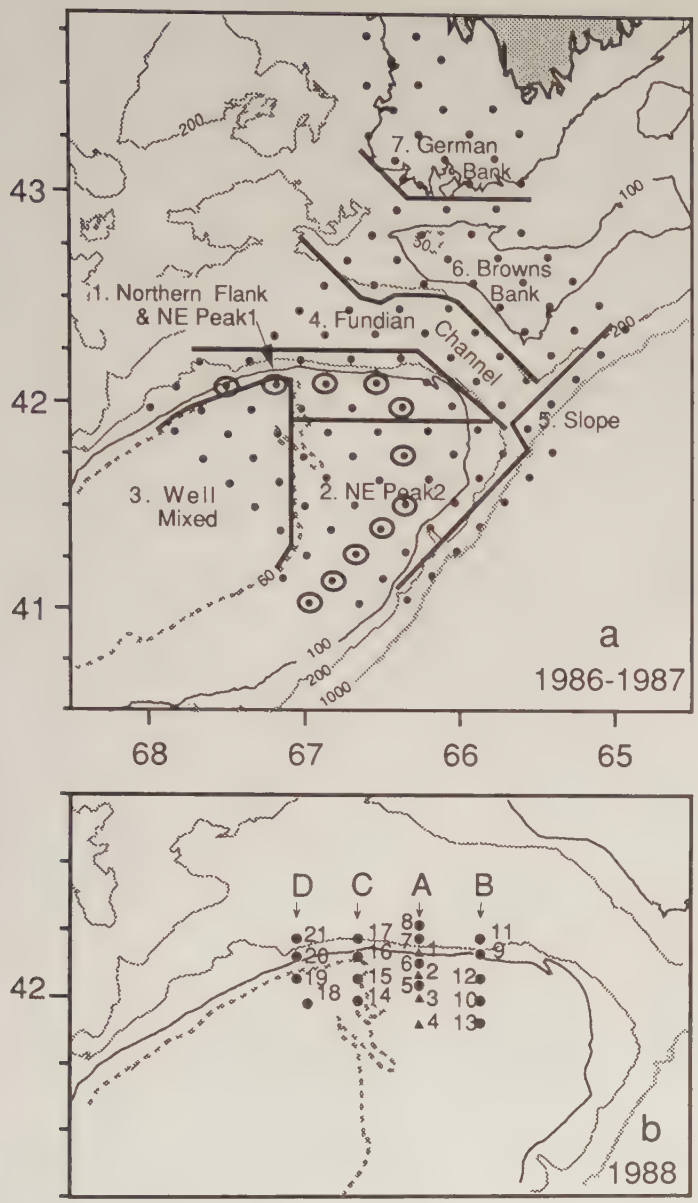

FIG. 4. Stations sampled for planktonic sea scallop larvae in (a) 1986 and 1987 and (b) 1988. Shown in Fig. 4a are areas for comparison of the abundance and length frequency of sea scallop larvae. Circled stations were used in an analysis of larval scallop length in relation to position on axis of the residential current (see text). Triangular symbols in Fig. 4b are stations where moored current meters were located.

1985, stations were sampled every 7 nautical miles on a series of transects from inshore to offshore on the Scotian Shelf, extending on to Georges Bank. Cruises in October 1986 and 1987 were based on a systematic survey grid with 10 nautical mile spacing (Fig. 4a). During these cruises the Georges Bank stations were sampled twice, and they are therefore divided into two periods (Table 2). Stations in September 1987 were those of a National Marine Fisheries Service plankton survey (MARMAP).

In 1988, sea scallop larvae were collected only at stations on Lines A to D on the northern flank of Georges Bank (Fig. 4b) as part of the Georges Bank Frontal Study. The physical oceanographic component included moored current meters at stations 1–4 on Line A, the area expected to encompass the maximum on- and off-bank excursions of the front based on previous studies.

Sampling

At most stations, sampling consisted of vertically integrated hauls with two plankton nets mounted on a bongo frame with two 40-cm-diameter openings. Each net was 156 cm in overall length, with a removeable PVC cod end bucket. The nets were fitted with NITEX mesh with openings of either 85 μm (1985–87) or 53 μm (1988). A difference in the sampling efficiency of the different meshes was accounted for (see below). The nets were hauled vertically at 0.5–0.8 $m \cdot s^{-1}$. Maximum tow depth was 5–10 m off bottom for bottom depths less than 100 m. For depths greater than 100 m the maximum tow depth was 200 m for October of 1985, 1986, and 1987 and ~100 m for other cruises (Tremblay 1991). Gear depth was measured by a mechanical or electronic meter block, with corrections for wire angle. The volume of water sampled was estimated from a TSK flowmeter that was mounted in the opening of at least one of the nets.

For two of the 10 transects in October 1988, a pump system (described in Tremblay and Sinclair 1990a) was used for sampling scallop larvae. The depth range sampled, 10–45 m, is where most sea scallop larvae are concentrated (Tremblay and Sinclair 1990a).

Samples were preserved in 4% formalin buffered with sodium borate. In the laboratory, scallop larvae and other heavier components of the plankton samples were physically separated from the lighter components using a colloidal silica (Tremblay et al. 1987). Sea scallop larvae in the samples were then identified and counted using a dissecting microscope. Subsamples were taken when the total number of larvae in the samples exceeded ~300. The sample was split with a Motoda splitter such that a total of at least 200 larvae were counted. After the larvae were transferred from the counting dish to a welled slide, their lengths were measured to the nearest 5.8 μm (half the width of the ocular micrometer units) using an inverted microscope.

Physical Data from the Georges Bank Frontal Study

Synoptic measurements of temperature, salinity, and currents across the frontal zone were available for the period September 29 to October 14, 1988. These data were obtained from the moored current meters on Line A (Fig. 4b) and were processed and made available by the Physical and Chemical Science Branch, Bedford Institute of Oceanography. Some of these data are tabulated in Loder and Pettipas (1991).

Data Analysis

The volume (cubic metres) measured by the plankton net flowmeters was used to standardize the larval abundance per square metre of sea surface as follows:

$$\text{No. of larvae} \cdot m^{-2} = \text{count/volume} \cdot \text{water column depth}$$

where water column depth = the lesser of the station depth, or 200 m. Estimation of sample volume when flowmeter readings were unavailable or spurious (about 10% of all tows) is documented in Tremblay (1991).

The length measurements of each sample were used to estimate the number per square metre within each 5.8-μm size class from 104 to 292 μm. Length frequencies of sea scallop larvae retained by an 85-μm net versus a 53-μm net indicated that the former was not effective at retaining larvae less than about 124 μm. Correction factors were developed based on simultaneous tows with 53- and 85-μm nets in 1988 (Tremblay 1991). Abundance estimates for larvae less than 124 μm based on

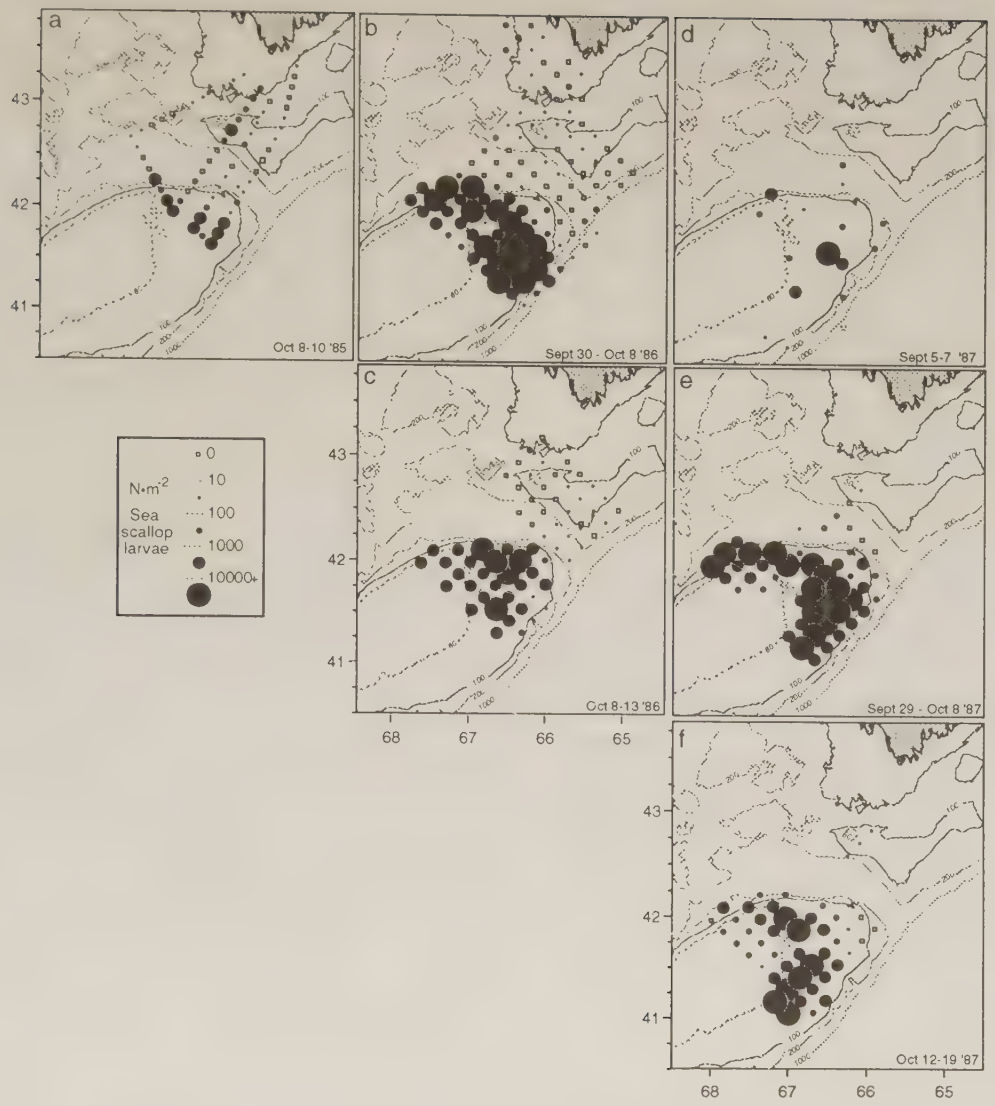

FIG. 5. Abundance of sea scallop larvae in the Georges Bank region as estimated by plankton surveys. Shown are expanding symbol plots of the number of sea scallop larvae per square metre for six sampling periods from 1985 to 1987. (a) 1985; (b and c) 1986; (d–f) 1987.

85-μm nets were increased by factors of 2.8 (120.8 μm), 15.8 (115 μm), and 22.4 (≤109 μm).

To compare the length frequency and abundance of larvae, the sampling area was divided into seven areas based on bathymetry and current structure (Fig. 4a). The first three areas are on Georges Bank: (1) northern flank and northeast peak 1, (2) northeast peak 2, and (3) well-mixed area (<50–60 m). The remaining four areas, sampled less intensively, are (4) Fundian Channel, (5) Slope southeast of Browns Bank and off Georges Bank, (6) Browns Bank, and (7) German Bank and Lurcher Shoals area. Overall length frequencies of sea scallop larvae within these areas were weighted by the abundance of larvae per square metre at each station.

Statistical analyses were performed using SYSTAT (Wilkinson 1987). Nonparametric statistics were used to compare the abundance of larvae among areas because of unequal variances (Tremblay 1991). To correct for the number of comparisons, the significance level was adjusted by the Bonferonni procedure, in which the pairwise significance level equals the overall significance level (0.05) divided by the number of comparisons (Wilkinson 1987; Neter et al. 1985).

Data Plotting

The spatial variation in the abundance (number per square metre) of sea scallop larvae is depicted with two types of plots: expanding symbols and shaded contours. The expanding symbols plots were used to depict the abundance of larvae over the whole sampling area during each sampling period. Shaded contour plots were used for examining the distribution of "patches" of sea scallop larvae on Georges Bank in 1986 and 1987, when the bank was sampled twice. In this type of plot, a surface is formed by defining triangles where the data points (number of scallop larvae per square metre) form the vertices of triangles connecting adjacent data points (Mohn et al. 1989). Because the shading at any one station is influenced by the surrounding stations, the time period of the data for any one plot was narrowed to increase its synopticity. For 1986, stations were excluded if they were not sampled within the periods October

TABLE 3. Abundance of sea scallop larvae in the Georges Bank region. Shown is mean and coefficient of variation (%, in parentheses) of the number of larvae·m^{-2} for areas depicted in Fig. 5. Mann–Whitney comparisons were done between areas within years. Areas followed by the same letter had distributions which were not significantly different using a p value of $0.05/N$, where N is the number of comparisons within the year. Areas: 1, Northern flank and northeast peak 1; 2, northeast peak 2; 3, well mixed; 4, Fundian Channel; 5, Slope southeast of Browns Bank and off Georges Bank; 6, Browns Bank; 7, German Bank and Lurcher Shoals.

	Survey period				
		1986		1987	
Area	1985 Oct. 8–10	Sept. 30 – Oct. 8	Oct. 8–13	Sept. 29 – Oct. 8	Oct. 12–19
1	1273 *ab* (152)	7929 *a* (123)	6868 *a* (87)	9069 *ab* (130)	2161 *a* (167)
2	2225 *a* (71)	20080 *a* (131)	4260 *a* (93)	16757 *a* (160)	6646 *a* (115)
3	—	3183 *a* (95)	2499 *a* (82)	1880 *bc* (155)	1201 *a* (105)
4	17 *c* (135)	2 *c* (200)	22 *b* (118)	169 *c* (158)	—
5	—	161 *b* (181)	—	—	—
6	240 *bc* (190)	40 *bc* (223)	5 *b* (140)	5[a] (140)	—
7	23 *c* (117)	63 *bc* (205)	—	—	—

[a]Insufficient number of stations for comparison.

2–6 and 8–11. Stations which were sampled twice in 1986 were separated by 4–6 d. For 1987 the two periods October 2–6 and 12–19, and stations sampled twice were separated by 10–15 d.

Growth of Sea Scallop Larvae in Hatcheries

To interpret the length frequency distributions of sea-caught larvae, it is necessary to have an estimate of the larval growth rate. Growth rates under different environmental conditions of temperature are currently unavailable (Tremblay 1988), so what follows is the typical development rate under hatchery conditions (12–15°C, ad libidum feeding) from a variety of sources (Culliney 1974; Couturier 1986; Hurley et al. 1987; Tremblay 1988). Sea scallop eggs are negatively buoyant, and the first swimming stage (trochophore) is reached 24–48 h after fertilization. The earliest shelled stage (D shape, or straight hinge) is ~105 μm in length and is reached after 3–4 d. Metamorphosis can occur after an additional 25–30 d, although some delay is possible. Prior to the size when sea scallop larvae are competent to metamorphose (~235 μm), a growth rate of 5–6 μm of shell length per day is implied. Given that most sea scallop larvae on Georges Bank are found within a temperature range of 10–13.5°C (Tremblay and Sinclair 1990a), the growth rate to metamorphosis is expected to be 4–6 μm·d^{-1} if the larvae are not food limited. If food limited, larvae would still increase in shell length but at a lower rate. Even during starvation the shells of scallop larvae can increase in length for a few days at 1–2 μm·d^{-1} (Tremblay 1991).

Results

Georges Bank versus Adjacent Areas

The mean number of sea scallop larvae per square metre was generally 10–100 times greater on Georges Bank than in the Fundian Channel, on the Slope, and on the Scotian Shelf (Fig. 4, 5; Table 3). Larvae were often absent from samples in the Fundian Channel. The greater abundance of scallop larvae on Georges Bank was statistically significant for each survey for which there were sufficient data for testing (Table 3). The mean number of larvae per square metre within the different areas was positively related to the fishery landings in Table 1 (Spearman rank correlation, $r = 0.88$, $N = 8$, $p < 0.01$).

A comparison of the length frequencies of larvae from Georges Bank, the Fundian Channel, and Browns Bank during 1985 and 1986 indicates some degree of separation among these areas. In 1985 (Fig. 6a–6c) the length frequency distribution of larvae on Georges Bank was statistically different from both the Fundian Channel and Browns Bank. In particular, larvae greater than ~207 μm were much less frequent on Browns Bank than on Georges Bank. In 1986 the length frequency distribution of larvae on Georges Bank again differed from that of the Fundian Channel, but not from Browns Bank. Small larvae (<124 μm) indicative of local spawning were present on Browns Bank during both 1985 and 1986 (Fig. 6c, 6f).

Spatial and Temporal Trends on Georges Bank

The abundance and length frequency distribution of larvae on Georges Bank in late summer indicate that spawning had begun recently (Fig. 5, 7). Sea scallop larvae were present in late August and early September (Fig. 5d), but larvae greater than about 200 μm were generally lacking at this time (Fig. 7c, 7f). Larvae were less abundant in early September 1987 (Fig. 5d) than in October (Fig. 5e, 5f), and peak spawning probably occurred around mid-September. By early to mid-October of 1987 and 1988, the length frequency distributions showed significant shifts to larger sizes (Fig. 7), but the presence of small larvae (<124 μm) estimated to be 6–10 d old indicates that spawning occurred as late as the second week of October (Fig. 7h). Compared with 1987 (Fig. 7c–7e), larger sea scallop larvae were more prevalent in all surveys in 1988 (Fig. 7f–7h), suggesting that spawning occurred earlier in the latter year, or that the growth rate was higher.

Significant differences in larval abundance among the areas on Georges Bank (Fig. 4) were few. Although a reduction in larval abundance was often apparent in the shallowest part of the well-mixed area, and on the extreme northeast part of the bank (Fig. 5), the mean number of sea scallop larvae per square metre was significantly lower only in the mixed area during the first part of October 1987 (Table 3).

To better relate the distribution of sea scallop larvae to that of adults, and to assess evidence for passive transport of larvae in the direction of the residual circulation, patches of sea scallop larvae in 1986 and 1987 are examined below.

Patches during the 1986 survey

The time separating the midpoints of the two sampling periods in 1986 was 5.5 d. Several dense patches (>10 000·m^{-2}) of larvae were present during both sampling periods (Fig. 8a, 8b). The elliptically shaped patches were

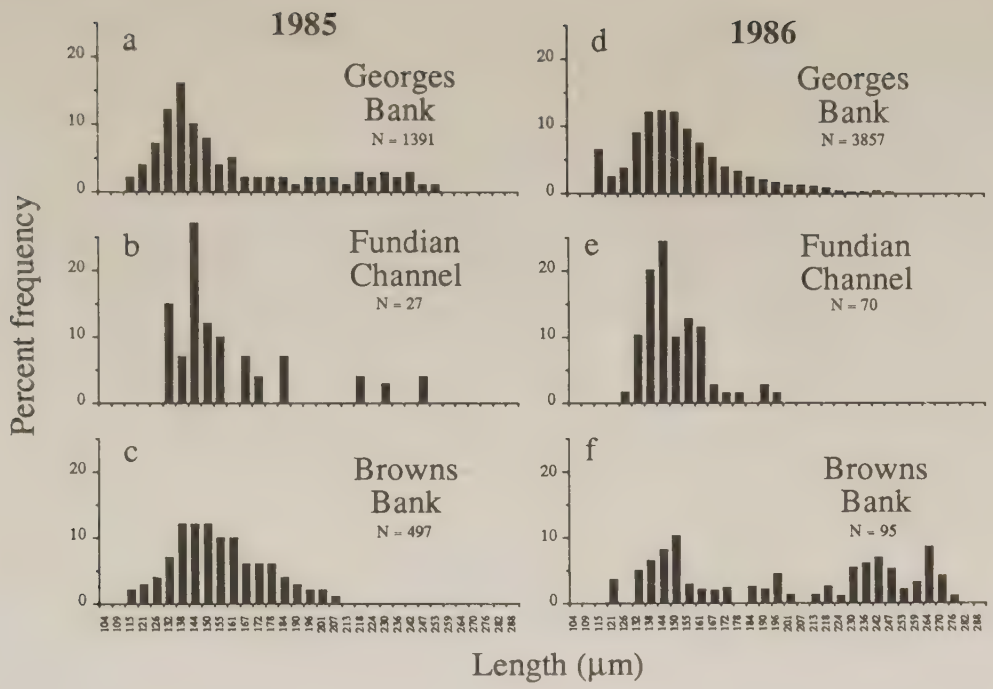

FIG. 6. Area differences in the size structure of sea scallop larvae in the Georges Bank region. (a–c) Georges Bank, the Fundian Channel, and Browns Bank in 1985; (d–f) Georges Bank, the Fundian Channel, and Browns Bank from September 30 to October 8, 1986. *N* is number of scallop larvae measured for each histogram. Within-year Kolmogorov Smirnov tests showed the following significant differences: a, b ($p < 0.001$); a, c ($p = 0.027$); d, e ($p < 0.001$); e, f ($p = 0.013$).

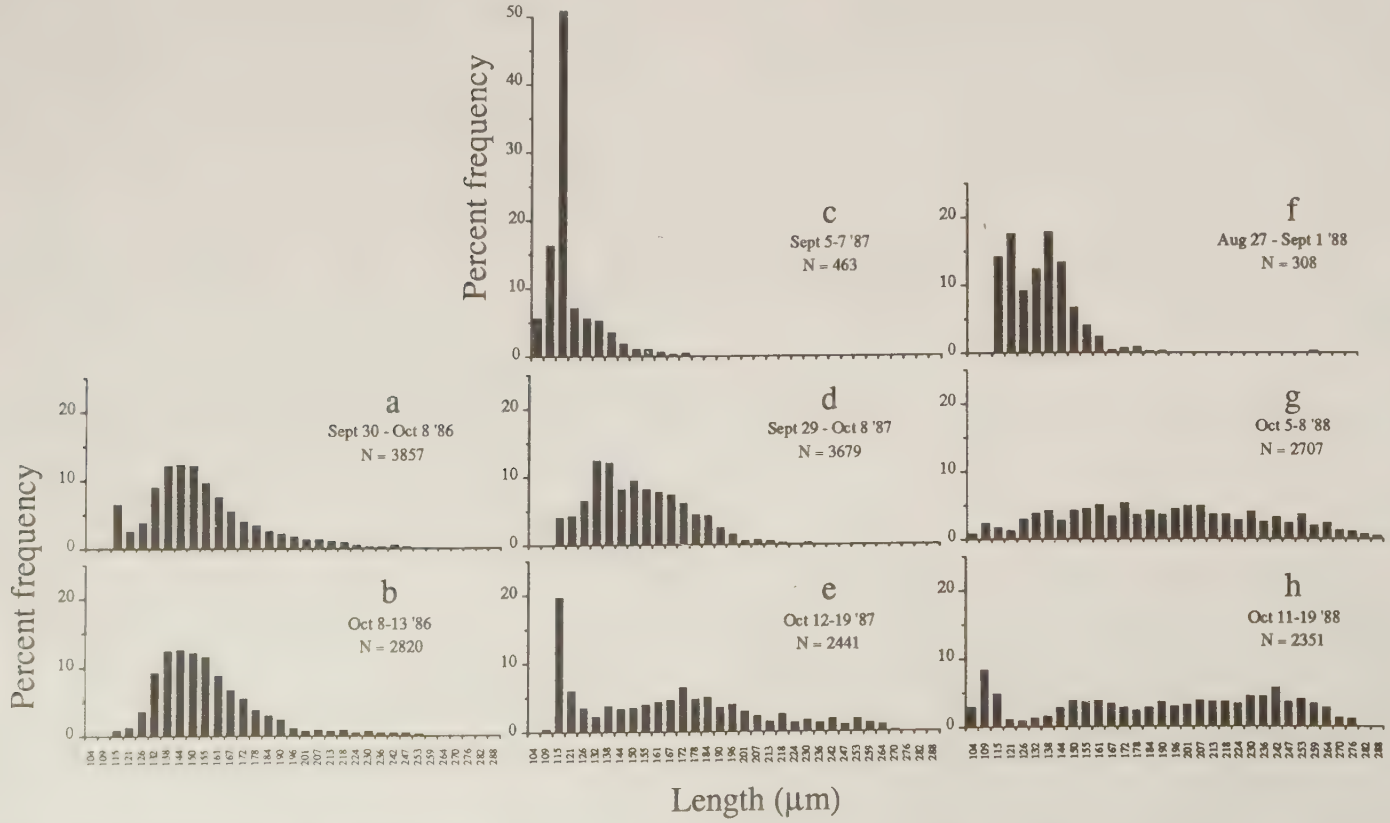

FIG. 7. Temporal changes in the length frequency of sea scallop larvae on Georges Bank. Number of measurements shown for each figure. (a and b) Northern flank, northeast peak, and well-mixed area during two sampling periods in 1986; (c–e) same areas during three sampling periods in 1987; (f–h) northern flank and part of northeast peak during three sampling periods in 1988. Within-year Kolmogorov Smirnov tests showed the following significant differences: d, e ($p < 0.001$); f, g ($p < 0.001$).

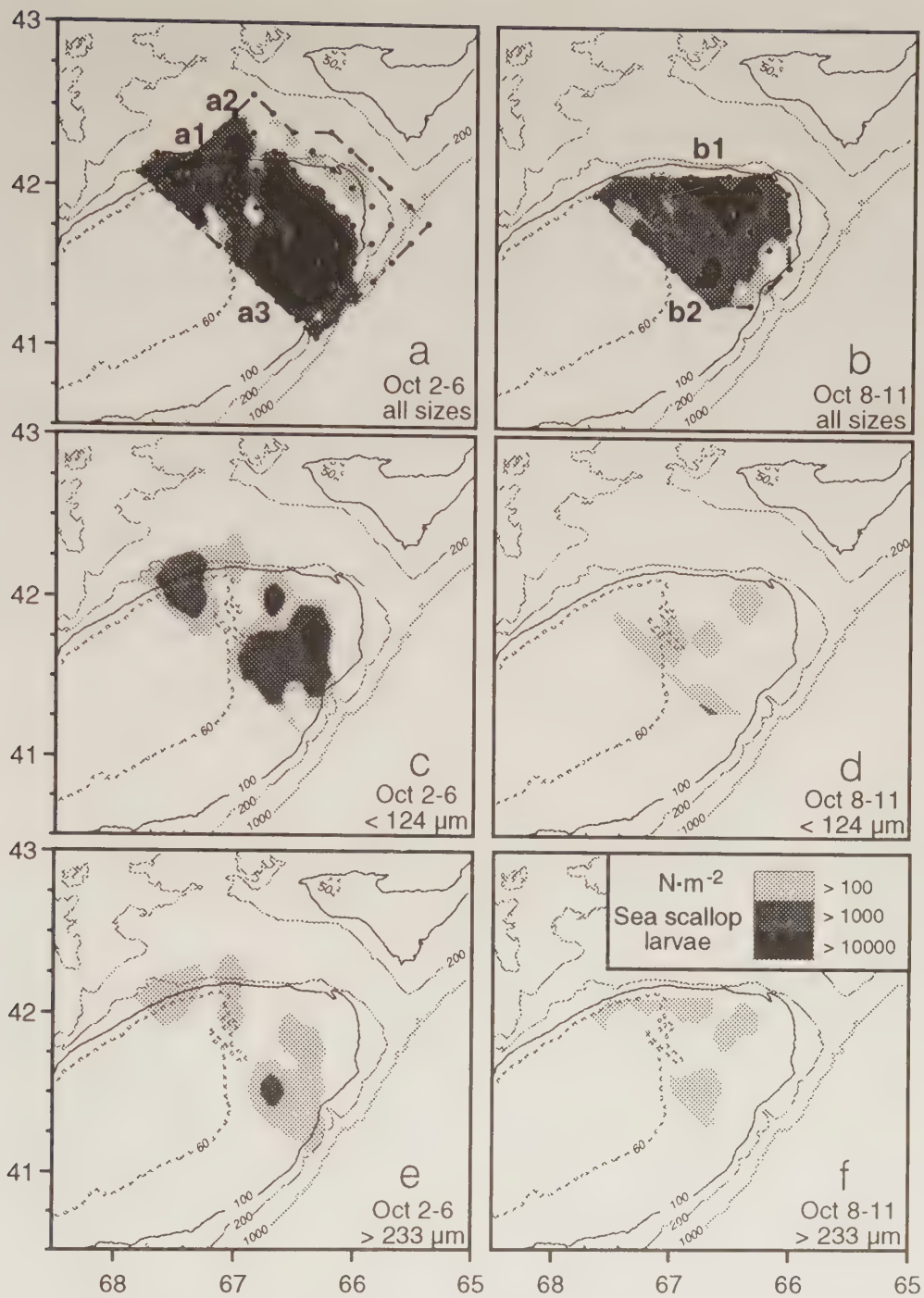

FIG. 8. Shaded contour plots of the abundance of sea scallop larvae during two sampling periods in 1986. (a and b) All sizes of larvae during the two periods; high-density patches of larvae (>10 000 larvae·m^{-2}) are labelled; shown are station locations (dots) and total area sampled (broken line); (c and d) small larvae (estimated to be 6–10 d old); (e and f) large larvae (capable of metamorphosis and settlement).

located mainly in areas with bottom depths of 60–100 m, but during the initial sampling period the patches extended beyond 200 m on the northern flank (Fig. 8a). Within each sampling period, small larvae (Fig. 8c, 8d) and large larvae (Fig. 8e, 8f) had concentrations in the same areas.

In relation to the concentrations of adults surveyed in 1986 (Fig. 2b), the patches of larvae were displaced more towards the centre of Georges Bank. Patches a1 and a2 (Fig. 8) were in U.S. waters (Fig. 1), which were not part of the Canadian adult scallop survey in 1986 (Fib. 2b).

There was considerable change in the distribution and extent of the larval patches between the two sampling periods (Fig. 8a, 8b). Patch b1, on the northern half of Georges Bank, was further to the east than patches a1 and a2, which were observed earlier. A patch in the position of b1 is consistent with the advection of patches a1 or a2 in the direction of the residual current during the period separating the two sampling periods (Fig. 3). The largest patch observed during the October 2–6 period (a3) was either much reduced or absent during the October 8–11 period.

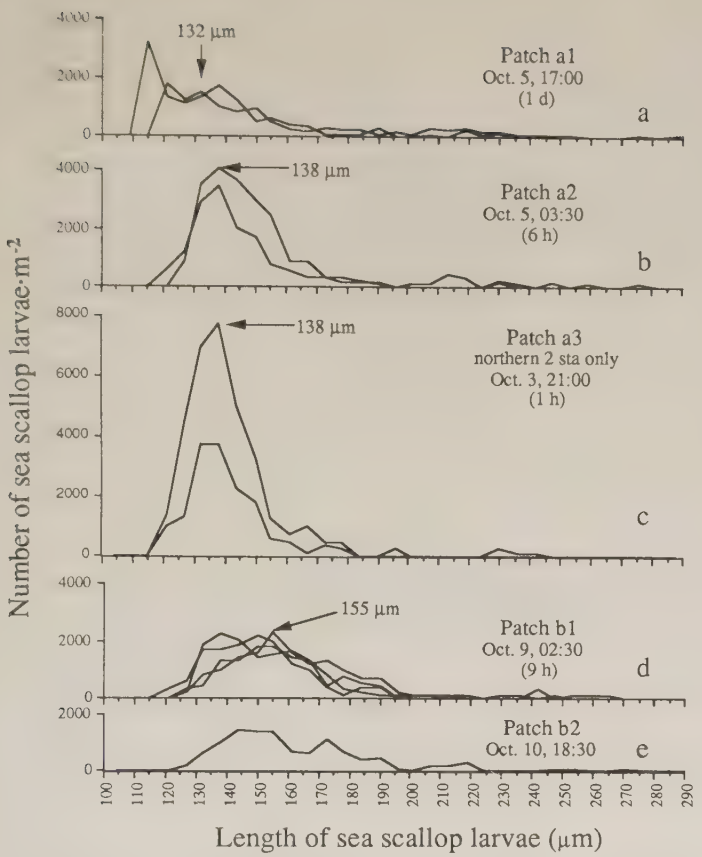

FIG. 9. Length frequency of sea scallop larvae at stations within high-density patches in 1986 (see Fig. 8). (a) Patch a1; text shows date and time of sampling midpoint of stations and time over which sampling occurred; (b–e) patches a2, a3, b1, and b2, with same information as given in Fig. 9a. For each patch the major length mode of the combined data is highlighted with an arrow. Combined length data from each patch were combined for Mann–Whitney tests between patches a1, a2, a3, and b2. Significant differences ($p < 0.05$) were as follows: a1, b1; a2, b1; a3, b1.

To explore the possibilities that patch b1 arose from movement of patches a1 or a2 in the direction of the residual circulation, or from patch a3, the length frequencies of larvae at stations comprising the patches were compared (Fig. 9). The lengths of larvae from patch b1 were statistically different from the larval lengths from each of patches a1, a2, and a3 (Mann–Whitney tests, Fig. 9). The length mode of patch b1 (155 μm) was 23 μm greater than the length mode of patch a1, and the time interval between the sampling of these two patches was 3.4 d (Fig. 9). This would suggest a larval growth rate of 6.8 $\mu m \cdot d^{-1}$, somewhat higher than the expected growth rate (4–6 $\mu m \cdot d^{-1}$; see section on hatchery growth). Compared with patch a1, the length mode of patch b1 was 17 μm greater, and the time interval between sampling patches a1 and b1 was 4 d. If, then, patch b1 and patch a1 are linked, the estimated larval growth rate would be 4.3 $\mu m \cdot d^{-1}$, which is within the expected range of growth rate.

For patch a3, the lengths of larvae from only the two stations closest to patch b1 were considered. The length mode of larvae at these stations was 138 μm, and the time separating the sampling of patches a3 and b1 was 5.2 d (Fig. 9c). If, then, patch b1 is a remnant of patch a3, a growth rate of 3.3 $\mu m \cdot d^{-1}$ is suggested, lower than that of hatchery-reared larvae.

Patches during the 1987 survey

The time separating the midpoints of the two sampling periods in 1987 was ~12 d, more than double that separating the two sampling periods in 1986, and station coverage was less than in 1986. For these reasons, no attempt is made to link the patches evident during the two sampling periods in 1987.

In general the distribution of sea scallop larvae in 1987 (Fig. 10a, 10b) was very similar to that of 1986. The distribution of larvae again overlapped that of the fished aggregations (Fig. 2c), but the larvae were more concentrated towards the centre of Georges Bank than were the adults. As in 1986, there was a reduction in patch size between the two sampling periods. Again the spatial distributions of different size groups of larvae were similar (Fig. 10e, 10f).

Unlike 1986, small larvae were widespread during the initial period in October 1987 (Fig. 10c) but were intensely concentrated in the southern most part of the study area between October 13 and 19 (Fig. 10d). This indicates that some scallop spawning occurred as late as the first week of October. In spite of this pulse of small larvae, the length frequency distributions of scallop larvae in 1987 (Fig. 7d, 7e) indicate a clear shift to larger sizes from early to mid-October. This shift is reflected by the increase in abundance of large larvae (>233 μm) between early and mid-October (Fig. 10e, 10f).

Lengths of Scallop Larvae along the Axis of the Residual Current

If there is transport of scallop larvae in the direction of the residual circulation, it would be expected that a synoptic survey would show an increase in larval length along the axis of the residual current. To test for such an increase, the median lengths of larvae were calculated for stations along the expected axis of the current (Fig. 4) for each of the two sampling periods in 1986 and 1987. A significant correlation between median length and distance was found only for the first sampling period in 1986 (Spearman rank correlation, $r = 0.92$, $N = 9$, $p < 0.01$).

Abundance of Different Sized Larvae

The mean numbers of sea scallop larvae per square metre in five size groups for Georges Bank (exclusive of the well-mixed area) are shown in Table 4. The smallest size group (<124 μm) was usually less abundant than the second size group (124–158 μm), suggesting that the abundance of the smallest larvae was undersampled. The last size group (>233 μm) includes larvae ~4–6 wk in age that are capable of metamorphosis and settlement. This size group had the lowest abundance, ranging from 44 to 497 $larvae \cdot m^{-2}$ (Table 4).

Larval Distribution across the Frontal Zone of the Northern Flank during October 1988

Physical conditions

Temperature sections from the moored instruments on Line A (Fig. 4b) for periods when sea scallop larvae were sampled are shown in Fig. 11. Larval transects took 2–10 h to complete, a time period over which the frontal zone can move appreciably, and therefore, two temperature sections are presented for each tidal period closest to the time of sampling: one when the frontal zone was at its most southerly, on-bank position and one when it was at its most northerly, off-bank position. The times corresponding to these maximum frontal excursions were determined from the current meter data at stations 2 and 3. When the hourly mean current changed from south to north the frontal zone was near its most southerly position; when it changed from north to south the frontal zone was near its most northerly position.

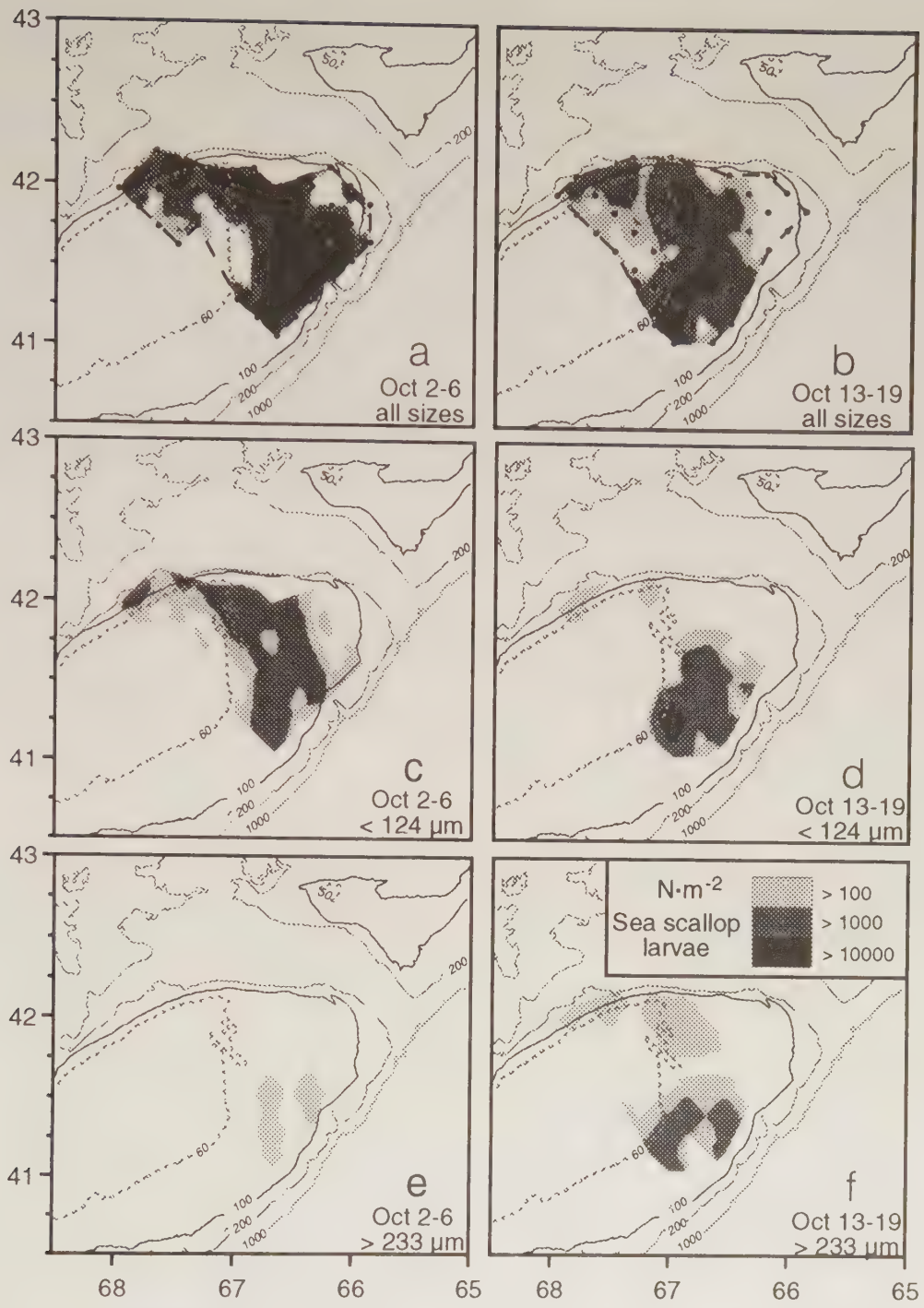

FIG. 10. Shaded contour plots of the abundance of sea scallop larvae during two sampling periods in 1987. (a and b) All sizes of larvae during the two periods; shown are station locations (dots) and total area sampled (broken line); (c and d) small larvae (estimated to be 6–10 d old); (e and f) large larvae (capable of metamorphosis and settlement).

There was no single isotherm that could be used to define the front, since the transition from mixed to stratified waters was very gradual (Fig. 11). In the upper 40–50 m, where sea scallop larvae are found, the horizontal temperature gradients were weak. A prominent feature in the hydrography of the northern flank during October was a short period of increased on-bank stratification. From October 5 to 9, colder water (<9°C) more typical of the Gulf of Maine occurred as far up on the bank as station 3 (Fig. 11a–11d), and differences due to tidal stage were small. During low on-bank stratification (after October 9), cold water was limited to the edge of the bank and differences due to tidal stage were greater (Fig. 11e–11j).

The temperature difference from near surface to ~64 m (Fig. 12a) provides a more detailed picture of the temporal change in on-bank stratification, including the advective effect of the semidiurnal tidal currents at higher frequencies. At a given location in the frontal zone, there is a semidiurnal cycle in temperature stratification as the position of the front is advected back and forth in a north–south direction. The higher on-bank stratification of October 5–9 coincided with several

TABLE 4. Abundance (no.·m^{-2}) of sea scallop larvae within different length groups on Georges Bank (northern flank and northeast peak 1 and 2; see Fig. 5).

Survey period	Length (μm)				
	<124	124–158	159–193	194–233	>233
Oct. 2–6, 1986	1342	8857	3927	1047	155
Oct. 8–11, 1986	103	3069	1548	256	86
Oct. 2–6, 1987	1343	9320	5520	515	44
Oct. 13–19, 1987	1493	895	1499	874	497

phenomena. First, two storms passed through the region resulting in strong winds from the north (Fig. 12b), and the north–south component of the residual current was in the on-bank direction (Fig. 12c). Second, October 4 was a neap tide, and tidal current speeds were reduced for several days before and after this date (Loder and Pettipas 1991). After October 8, northerly winds subsided, and on-bank stratification became progressively less.

Distribution of sea scallop larvae across the frontal zone

Seven of the 10 transects for larval scallops (Table 5) continued more than 1 km north of the bank edge (Fig. 13). On six of these, the relative abundance of sea scallop larvae declined at the bank edge (Fig. 13b, 13e) or at the outer most slope station (Fig. 13a, 13f–13h). Repeat transects of Line A over approximately two tidal cycles (Fig. 13f, 13g) indicate that "smearing" of abundance patterns was not a major problem. For stations sampled on each of these two transects, larval abundance was significantly correlated (Pearsons correlation coefficient, $r = 0.85$, $N = 6$, $p < 0.05$).

During the period of high on-bank stratification (October 5–8), sea scallop larvae peaked in abundance on the bank within 7 km of the bank edge (200-m contour) on each of the five transects (Fig. 13a–13e). Compared with Lines A and B, the abundance peaks on the upstream Lines C and D were closer to the bank edge. It is not known how abundance changed beyond the bank edge on Lines C and D, since sampling did not continue into the deep water. During the period of low on-bank stratification (after October 9), peaks in the number of sea scallop larvae (per square metre, or per cubic metre in the case of pump samples) were less prevalent and not associated exclusively with the stations near the bank edge (Fig. 13f–13j). Larval patchiness on the scale of the transects appeared to be lower: the coefficient of variation (CV) of station abundances for any one transect ranged from 45 to 96% after October 9 compared with 70 to 110% for the five transects from October 5–8 (Table 5).

Only for Line A are data available to relate the cross-bank gradient in temperature and current speed to larval abundance. There was no association between the vertical temperature gradient and relative larval abundance (Fig. 14a). Abundance peaks during the October 5–8 period occurred where the vertical temperature gradient from 10 to 60 m was almost as great as at the deepest station (5–7°C, Fig. 11a–11d). There was, however, a significant relationship between larval abundance and the speed of the along-front (east–west) component of the current. For any one transect, scallop larvae tended to be more abundant where the along-front current speed (decimated to 6 h to remove the tidal component) was greater (Fig. 14b).

A broad size range of larvae was present at all stations, and the length frequency distributions tended to be polymodal (Fig. 15). To examine whether there were cross-bank trends in

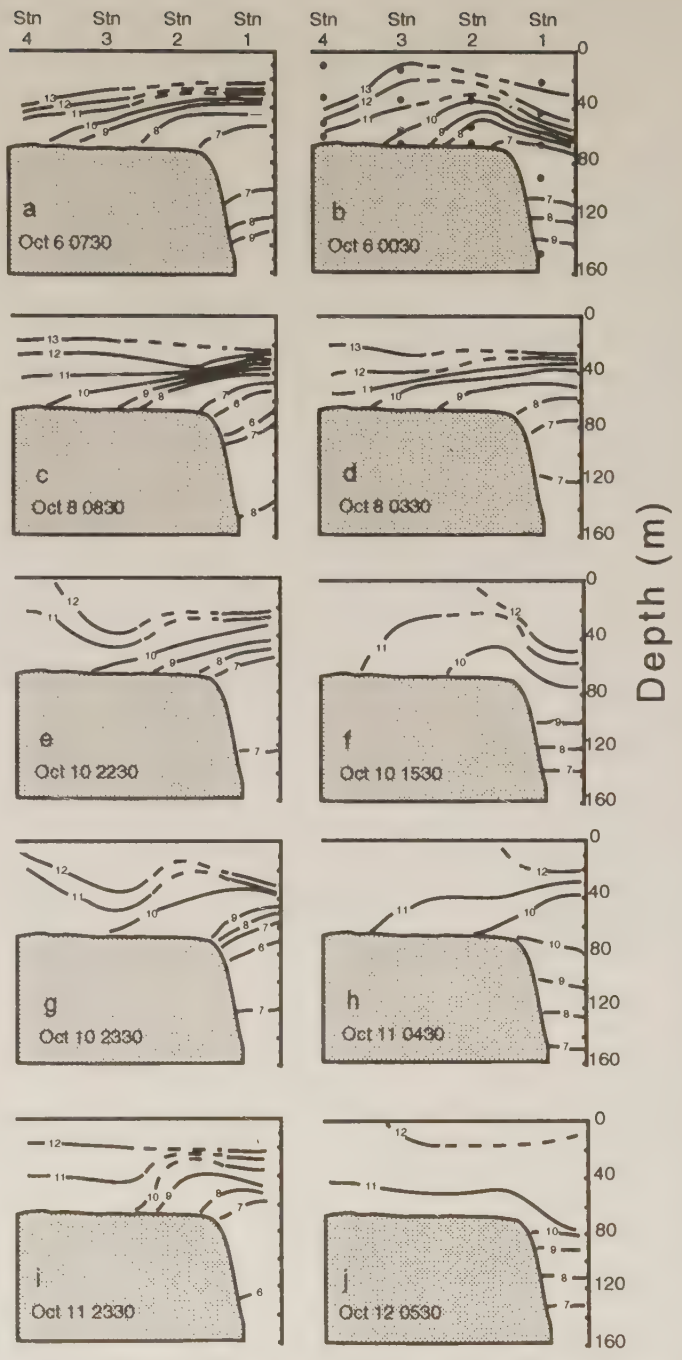

FIG. 11. Temperature (°C) sections on Line A (see Fig. 5b) during October 1988. Left and right panels show extremes in frontal position during a tidal cycle (see text). The front is in the maximum on-bank position in sections on left, and in maximum off-bank position in sections on right. Depths of current meters indicated by solid circles in Fig. 11b.

larval length, the 25th, 50th, and 75th percentiles of larval length were computed for each of the 56 stations. Transect data were then grouped based on the on-bank stratification characteristics during sampling: high stratification, transects 1–5; intermediate stratification, transects 6 and 7; and low stratification, transects 8–10. For transects 1–5, a curvilinear relationship was apparent: larvae were smallest at the mid transect stations (Fig. 16a; Table 6). For transects 6 and 7, larvae tended to decrease in size in the off-bank direction (Fig. 16b; Table 6). For transects 8–10, larvae increased in size in the off-bank direction (Fig. 16c; Table 6) due to a pulse of very small larvae

TABLE 5. Abundance and patchiness of sea scallop larvae across the frontal zone of the northern flank. Shown for each transect is degree of temperature stratification (H = high; L = low), peak abundance, and coefficient of variation (CV) of abundances for all stations on the transect.

Transect No.	Line No.	No. of stations	Date	On-bank stratification	Abundance at peak (no.·m^{-2})	CV
1	A	7	Oct. 5–6	H	15615	102.2
2	B	5	Oct. 6	H	2596	83.7
3	D	3	Oct. 7	H	11212	91.0
4	C	3	Oct. 7	H	33512	109.6
5	A	5	Oct. 8	H	17687	69.1
6	A	8	Oct. 10	L	11160[a]	44.9
7	A	7	Oct. 10–11	L	8490[a]	55.7
8	A	8	Oct. 12	L	34898	95.7
9	C	4	Oct. 12	L	14803	47.2
10	A	7	Oct. 19	L[b]	9877	50.8

[a]Estimated from integrated pump samples between 10 and 40 m.
[b]Stratification assumed to be low for transect 10; temperature data were unavailable.

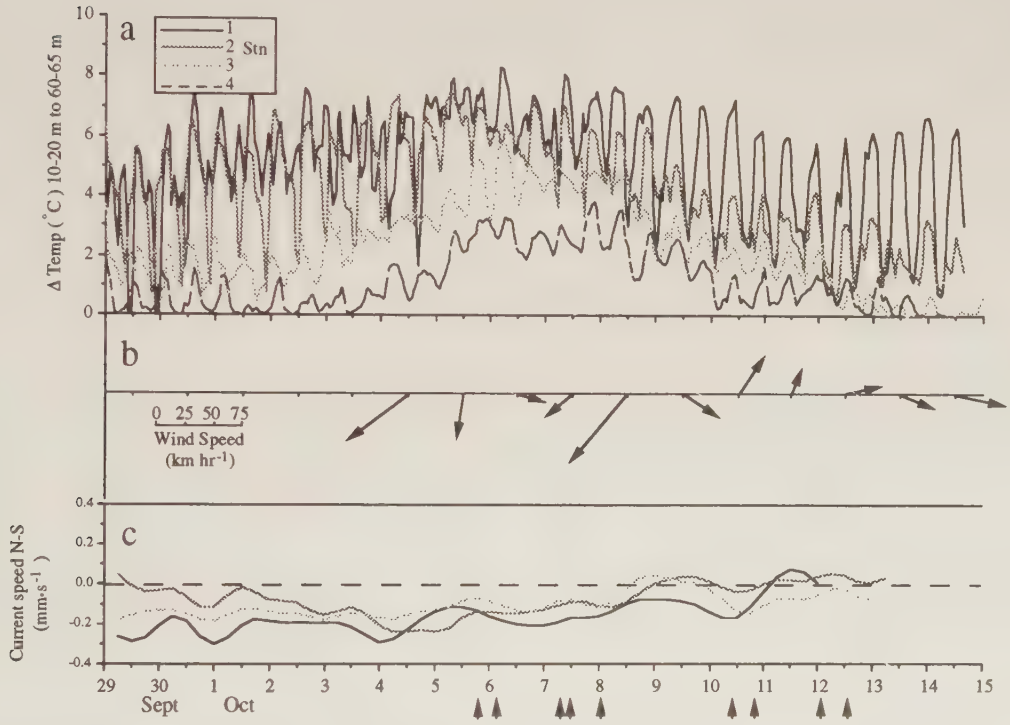

FIG. 12. Temporal changes in physical conditions during the 1988 sampling of sea scallop larvae in the Georges Bank frontal zone. (a) Hourly changes in temperature gradient from near surface to near bottom for moorings at stations 1–4; (b) mean daily wind speed and direction from the log of the RV *Lady Hammond*; (c) cross-bank current with tidal component removed (a negative current indicates on-bank flow). From moorings at stations 1 (21 m), 2 (35 m), and 3 (34 m). Arrows at bottom indicate when scallop larvae were collected on transects 1–10.

at the shallowest stations. Thus there was no consistent pattern in the cross-bank distribution of larval size.

Discussion

Scallop Spawning Time on Georges Bank and Seasonal Changes in Physical Retention

Since scallop eggs are externally fertilized, both mate proximity and spawning synchrony may influence reproductive success and ultimately recruitment (Sastry 1979; Langton et al. 1987; Paulet et al. 1988; Caddy 1989). Individual beds of sea scallops on Georges Bank can at times release most gametes almost synchronously (Posgay and Norman 1958). Some evidence for this from the current study comes from the dense, highly localized concentrations of small larvae during some surveys (Fig. 8c, 10d), but the scale of this study cannot resolve how synchronous spawning is within beds. Scallops on Georges Bank may "dribble spawn" as in coastal Newfoundland (Naidu 1970) and Maine (Langton et al. 1987), or may spawn synchronously more than once. Depending on seasonal variability in factors such as temperature, circulation, food, and predation, these different spawning patterns will increase or decrease the probability of larval survival.

Shifts in the size frequency distribution and abundance of larvae on the northeastern part of Georges Bank in 1987 and 1988 (Fig. 7) indicate that peak spawning was in the second

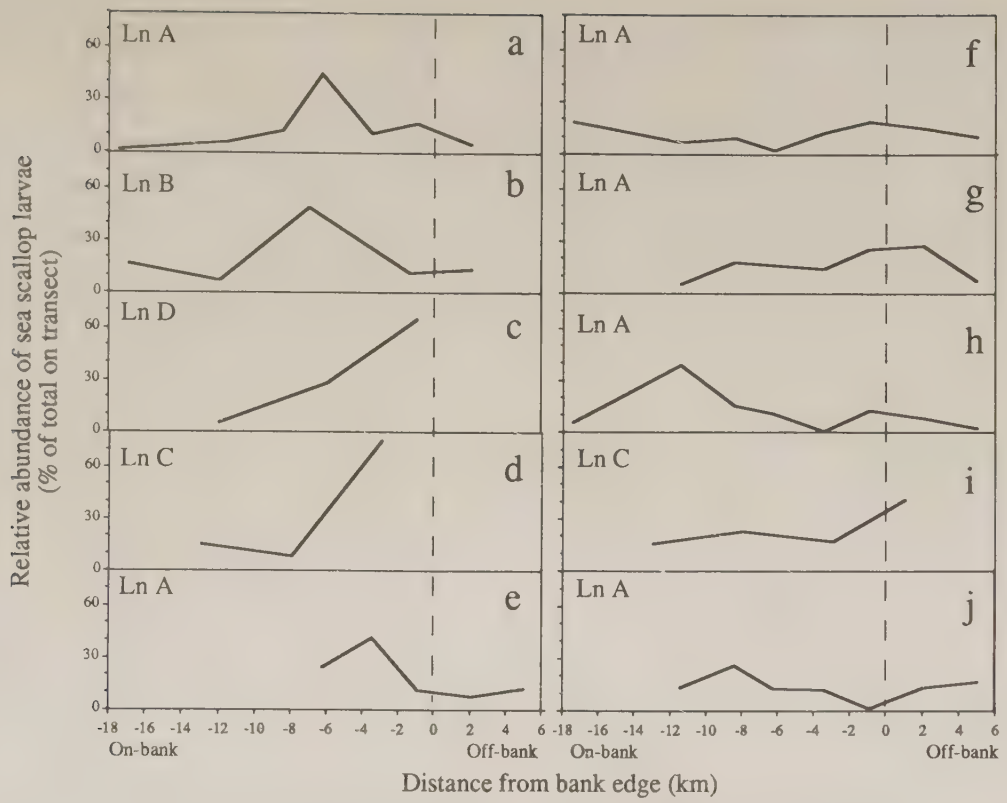

FIG. 13. Relative abundance of sea scallop larvae for each of 10 transects (a–j) of the northern flank. Line (Ln) identifier is indicated for each transect. Station positions are related to distance from the bank edge (200-m contour), which is shown by the vertical broken line.

half of September, but that some spawning occurred as early as mid-August and as late as early October. The major spawning of most other populations of sea scallops also occurs during late summer and fall (Dickie 1955; Naidu 1970; Robinson et al. 1981; MacDonald 1984), but an additional early summer spawning occurs in some populations (Naidu 1970; Savage 1980). On Georges Bank, the seasonal trend in sea scallop gonadosomatic indices indicates that an earlier spawning occurs regularly, but its intensity is more variable than the fall spawning (G. Robert, Department of Fisheries and Oceans, Halifax, N.S., pers. comm.). The relative importance of the two spawning periods to recruitment on Georges Bank is an open question (Dibacco 1991).

The fall spawning of sea scallops on Georges Bank coincides with the period when retention and survival of larvae should be maximized. At this time of year, passive particles in the central, well-mixed area of the bank will remain the longest (>60 d), while those on the periphery of the bank will generally not remain as long (Loder et al. 1982; Colton and Anderson 1983; Loder et al. 1988). Maximum around-bank flow also occurs in late summer and early fall (Butman et al. 1982), when the recirculation time of drifters deployed at 10 m is estimated to be 33–55 d (Loder et al. 1988). Temperature on Georges Bank in autumn is also near the annual maximum, which will shorten larval development and the period of the life history when mortality rates are presumably at their highest. A coincidence between the timing of the larval period, warm temperatures, and high physical retention was also noted for Bay of Fundy sea scallops by Dickie (1955). He suggested that good recruitment to the fishery occurred if temperatures during the larval period were higher than average, increasing both larval retention and survival.

Exchange of Sea Scallop Larvae between Georges Bank and the Scotian Shelf

Sea scallop larvae on the northeast part of Georges Bank appear to be largely retained on the bank. Few larvae were found in the Fundian Channel. Plankton samples extended down to 200 m here, and larvae would have been collected if present. On the northern flank, dense patches of sea scallop larvae do extend beyond the edge of the bank (200 m), but such patches are not necessarily lost, since the front moves back and forth in this area, and the mean cross-bank circulation (north–south) is directed on-bank (Fig. 12c). Station coverage in the slope waters south of the bank was low, but larval abundance generally declined beyond the 100-m contour of the Southern Flank (Fig. 5b–5f). Sampling in the deep water north of the bank west of about 67° longitude was too sparse to evaluate retention for that part of the bank.

Other evidence indicating limited export of larvae from Georges Bank is the length frequency distribution of larvae, which showed differences among Georges Bank, the Fundian Channel, and Browns Bank. If there were continuous and extensive losses of scallop larvae from Georges Bank, the length frequencies of larvae in these three areas would be expected to be more homogeneous. The positive correlation between larval abundance and fishery landings (an index of spawning biomass) does not in itself indicate larval retention on Georges Bank, but shows that Georges Bank is not flooding the southern Scotian Shelf with larvae — in this extreme case, there would be no correlation between larval abundance and the index of spawning biomass. Inevitably some scallop larvae are lost from Georges Bank, but taken together, the evidence indicates that for the northeast part, these losses are small.

Several studies have suggested that larval behavior may be involved in the retention of fish larvae on Georges Bank or the

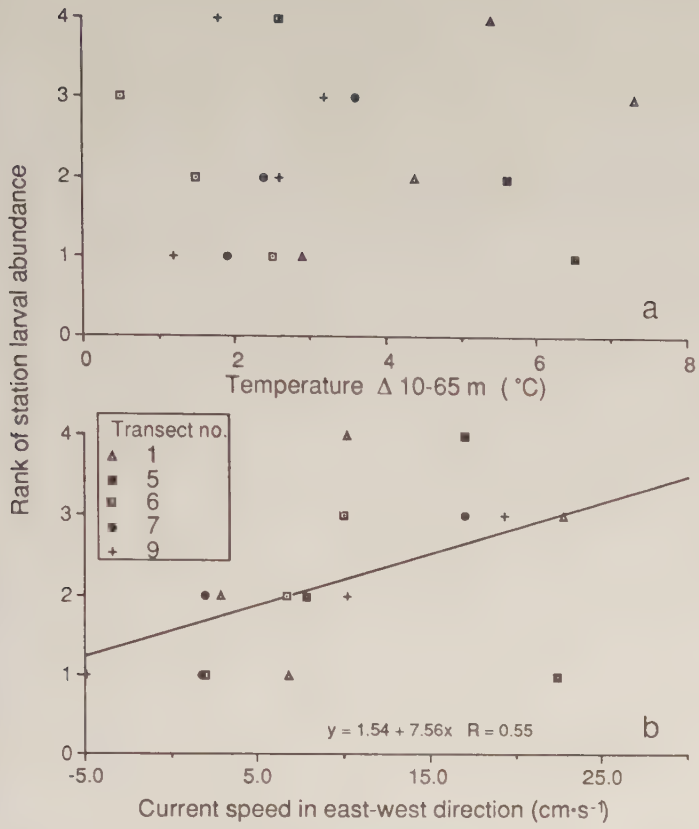

FIG. 14. Trends in the abundance of scallop larvae on Line A versus physical features. For each transect of Line A, stations with moored current meters were ranked based on larval abundance. Ranks were then plotted against (a) temperature difference between 10 and ~65 m and (b) current speed at the intermediate depth (34–45 m). Only in Fig. 14b was the Pearsons correlation coefficient significant ($p < 0.05$).

surrounding area (Iles 1971; Iles and Sinclair 1982; Stephenson and Power 1988; Lough and Bolz 1989). It might be expected that if vertical swimming behavior is important to the retention of fish larvae on Georges Bank, sea scallop larvae should be retained to a lesser extent. Swimming speeds of sea scallop larvae (<1.7 mm·s^{-1}; Silva and O'Dor 1988) are low compared with those of fish larvae. Small herring larvae, for example (~10 mm in length), can cruise at 10 mm·s^{-1} and have maximum speeds of 30 mm·s^{-1} (Blaxter 1965).

The fact that sea scallop larvae are associated with the pycnocline rather than near the surface (Tremblay and Sinclair 1990a) may enhance retention because current speed declines with depth. However, sea scallop larvae do not appear to "use" the vertical shear through vertical migration (Tremblay and Sinclair 1990a). If scallop larvae do vertically migrate on Georges Bank, the amplitude is less than about 10 m and is probably diel rather than tidal (Tremblay and Sinclair 1990b). Vertical migration of sea scallop larvae is thus unlikely to strongly affect their dispersal pattern, and the physical retentiveness of Georges Bank (Loder et al. 1988) is probably the primary factor determining the retention of scallop larvae there. Swimming behavior of other more mobile larvae may enhance retention, but such behavior is probably of secondary importance. Selection for behaviors which enhance retention of planktonic larvae (Burton and Feldman 1982) may not be necessary for areas such as Georges Bank, which have strong physical retention.

Dispersal of Sea Scallop Larvae on Georges Bank

Transport of scallop larvae in the direction of the residual circulation on Georges Bank is probable given (i) the strong residual circulation on the northern flank and (ii) sea scallop larvae do not undertake extensive vertical migrations to utilize current shear. However, evidence that such transport occurred during the present study is limited. Consideration of possible links between patches of larvae present during successive sampling periods in 1986 (Fig. 8) suggests the movement of patch a2 in the direction of the current, but other interpretations are possible. The significant increase in larval length along the expected axis of the current during the first survey in 1986 is consistent with larval transport, but the length increase was not seen in the other three sampling periods. High concentrations of larvae in the northwestern part of the sampling area (Fig. 8, 10) are also suggestive of larval transport, since adult scallop concentrations are thought to be low there (Fig. 2a), and strong residual currents are directed eastward (Fig. 3). Larvae there may have originated upstream, in the South Channel, or on the southeast part of the Georges Bank.

The more on-bank distribution of scallop larvae (Fig. 8, 10) relative to the adults (Fig. 2) suggests that some scallop larvae are transported to shallower parts of the northeast peak. If so, these larvae may subsequently be transported less, since currents are lower there (Fig. 3).

The evidence linking patch movements, larval lengths, and the residual circulation may be as strong as can be expected from a semisynoptic survey given that (i) groups of larvae appear to be spawned at different times and in different areas of Georges Bank (Fig. 8c, 8d, 10c, 10d) making tracking of individual patches difficult and (ii) the residual circulation (Fig. 3) represents mean conditions, and storms can result in short periods when the circulation is markedly different.

The fact that different sized larvae were distributed similarly (Fig. 8, 10) indicates that the factors affecting spatial distribution are not size specific and that differences in time and place of larval origin are secondary to the processes which affect distribution one to several weeks after spawning. Physical processes may aggregate all larvae in some way, or the survival rate of larvae may be greater in certain areas of Georges Bank. A physical aggregation mechanism is consistent with observations that different sized scallop larvae have similar vertical distributions (Tremblay and Sinclair 1990a) and thus should be subject to the same currents.

The lower abundance of scallop larvae in the centre of the well-mixed area (Fig. 5) is probably a result of the lower number of adults there producing fewer larvae, but lower larval survival cannot be ruled out. Although food concentrations (chlorophyll *a* <15 μm) are no lower in the well-mixed area (Tremblay and Sinclair 1990a), higher suspended sediment loads there (due to greater vertical mixing rates) may adversely affect larval feeding.

Northern Flank Frontal Zone and the Distribution of Scallop Larvae

During October, the frontal zone on the northern flank of Georges Bank is not as well defined as in summer. In addition, density stratification on the northern flank during October is subject to considerable variation over periods of a few days, at least in part due to wind events (Fig. 12). Neap tides and the associated reduced tidal currents which coincided with sampling in early October may have contributed to a reduction in the extent of the mixed area, thus increasing on-bank stratifi-

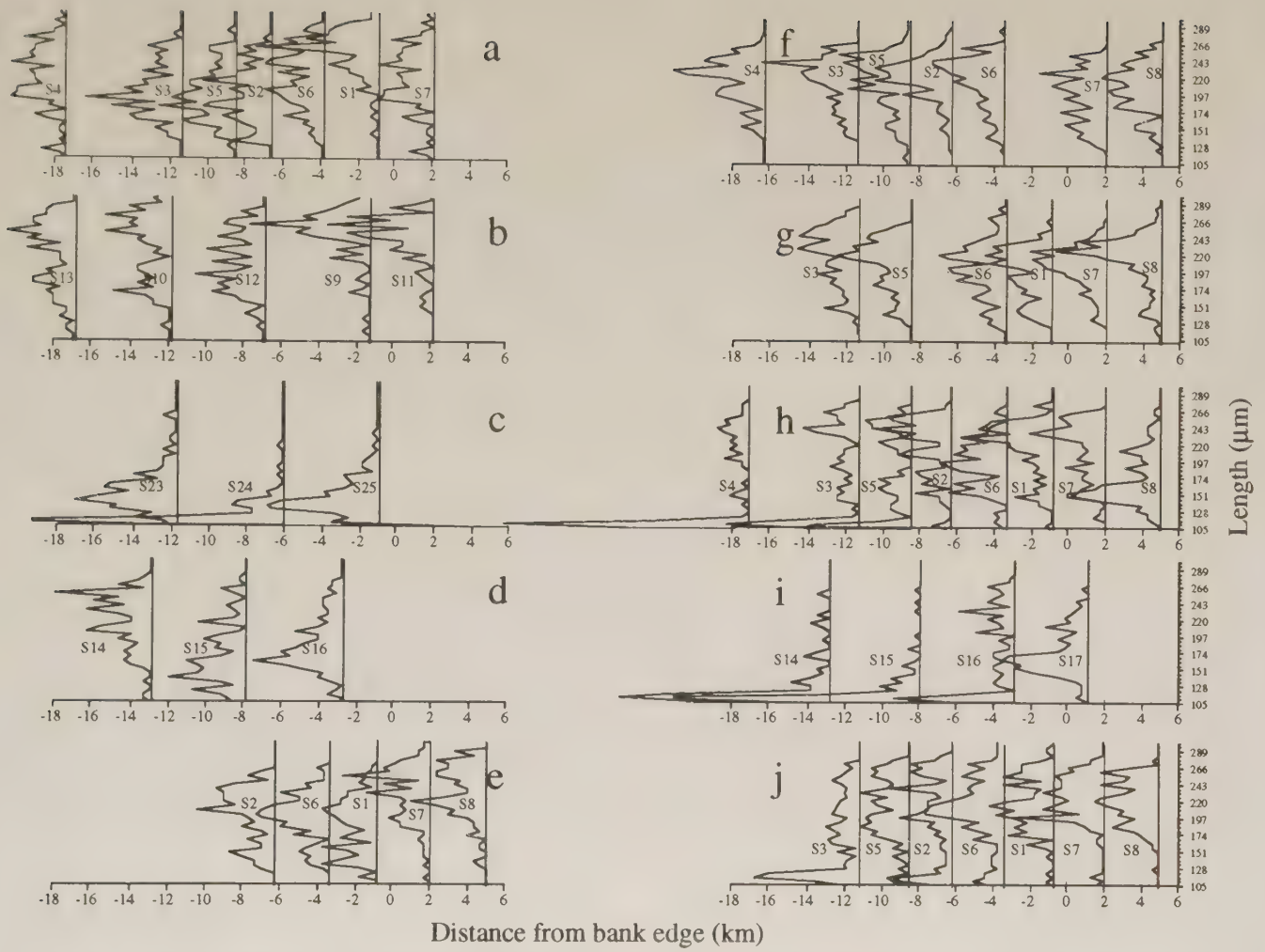

FIG. 15. Length frequency distributions for sea scallop larvae on 10 transects (a–j) of the northern flank. Station (S) positions are related to distance from the bank edge (200-m contour). Shown for each station is percent length frequency.

cation. However, on-bank stratification during September neap tides was markedly lower than in early October (J. W. Loder et al., in prep.), and thus the winds from the north must have contributed to the relatively high on-bank stratification of the latter period.

The reduced abundance of larvae north of the bank edge is consistent with the broadscale surveys of 1985–87, which collected few larvae within the Fundian Channel. The decline results from a lack of off-bank larval production, coupled with limited loss of larvae from the northern flank.

The peaks in larval abundance (and associated increased larval patchiness) on Line A occurred where the vertical temperature gradient was ~6°C (Fig. 14). This is equivalent to a vertical density gradient of greater than 1.5 sigma-t units, which is on the stratified side of the frontal zone using the criterion of Horne et al. (1989; 0.2–1.5 sigma-t units). Possible explanations for the peaks in larval abundance close to the shelf edge include those related to physical mechanisms alone, those which invoke larval behavior, and those related to increased survival of larvae. Larval behavior is unlikely for reasons stated earlier. Evidence for physical convergence of scallop larval is the positive relationship between relative abundance of scallop larvae and average current (Fig. 14). Convergence is an important feature of frontal zones (Simpson 1981) and is known to occur on Georges Bank earlier in the year. Northerly winds may have contributed to the larval abundance peaks during high on-bank stratification by pushing water and larvae up onto the bank.

Enhanced survival of larvae on the stratified side of the front due to increased food availability or reduced predation might also explain the abundance peaks. For these mechanisms to operate, cross-frontal exchange would have to be sufficiently low for increased survival rates to have a measurable effect on abundance.

Differences in the length of scallop larvae across the frontal zone (Fig. 16; Table 6) suggest that physical exchange across the zone is limited because rapid exchange should homogenize the size structure. Local larval production on the bank clearly leads to some variation in length composition across the frontal zone (Fig. 15h, 15i). Variation in size structure across the frontal zone may also result from the horizontal current shear and from the upstream origin of patches of larvae along the bank edge.

Physical models of the frontal circulation on the northern flank are necessary to evaluate whether the on-bank retention of sea scallop larvae, the periodic increases in larval patchiness, and the cross-bank differences on larval length can be explained in terms of the physical circulation or whether biological factors such as variation in larval survival are important. Further field

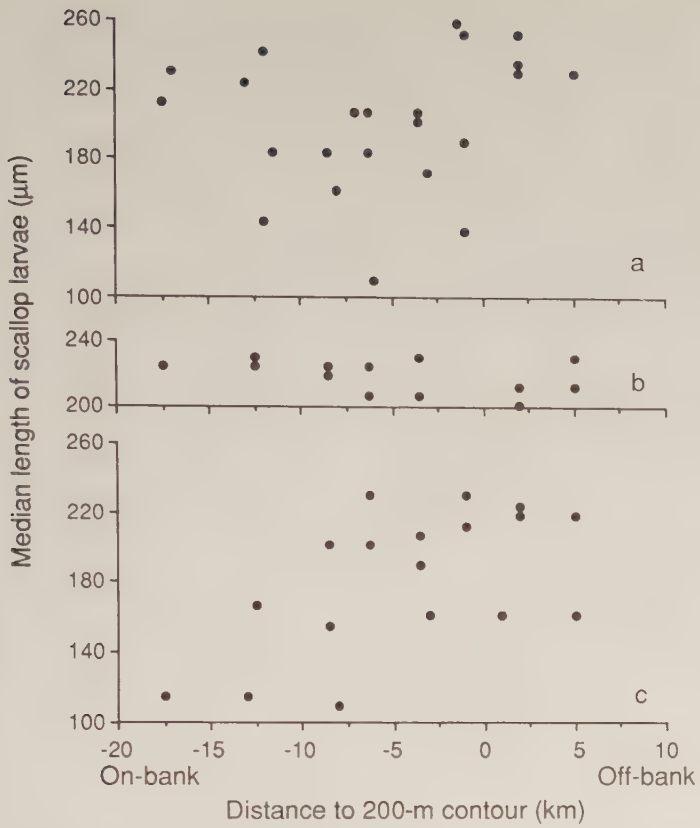

FIG. 16. Plots of median lengths of scallop larvae in relation to cross-bank position. Each point is from one sample at one station. (a) Transects 1–5; (b) transects 6 and 7; (c) transects 8–10. Correlation coefficients are shown in Table 6.

TABLE 6. Larval scallop length in relation to cross-bank position. Shown are correlation coefficients between length percentiles and distance from the 200-m contour for three groups of transects. The correlation coefficients for transects 1–5 are η, a measure of nonlinear association (Yule and Kendall 1950). For other transects, Pearsons correlation coefficient shown. $*p < 0.05$; $**p < 0.01$.

Transects	No. of stations	Percentiles		
		25th	50th (median)	75th
1 to 5	23	0.34	0.54*	0.49*
6 to 7	14	−0.19	−0.38	−0.75**
8 to 10	19	−0.66**	0.58**	0.31

applications of a lipid-based condition index (Gallager et al. 1986; Tremblay 1991) are needed to test a hypothesis related to enhanced larval survival in the area of the abundance peaks.

Implications for Population Structure and the Recruitment Process

Given that at least some scallop larvae are transported in the direction of the residual circulation, exchange among the scallop aggregations on Georges Bank (the northeast peak, the southeast part, and the South Channel) must occur. Quantifying this exchange is not possible from the present study, but should be a goal of future studies because this species is currently managed differently on the Canadian and U.S. sides of Georges Bank.

Transport of larvae in the direction of the residual circulation does not exclude the possibility that the productive northeast peak is largely self-sustaining, since scallop larvae that originate locally could seed this area in two ways. Some larvae may make a complete circuit of the bank before returning to the northeast peak to settle. Given the circulation time of 33–55 d and a larval period of 4–6 wk, this scenario is probable. Other larvae that originate on (or are transported to) the shallower part of the northeast peak may be displaced only small distances over the larval period because of lower residual currents. Complex larval dispersal patterns such as these are also thought to occur elsewhere in the Gulf of Marine area. In the Bay of Fundy, a gyre in the outer part of the bay appears to retain some scallop larvae, while others are transported to the inner bay (Tremblay and Sinclair 1988). Haddock larvae on Browns Bank can be either physically retained or transported to the inner Scotian Shelf (Campana et al. 1989).

The concept that some larvae which originate on the northeast peak eventually settle there, while others are lost from the area, is supported by an examination of stock and recruitment relationships among the three fished aggregations on Georges Bank (McGarvey 1991). McGarvey found that the strongest positive relationship between egg production and recruitment to age 2 was for the northeast peak, suggesting that this area is largely self-sustaining. McGarvey also found a positive relationship between egg production on the northeast peak and recruitment to age 2 on the southeast part of Georges Bank, suggesting that a large proportion of the larvae which seed the southeast part originate on the northeast peak.

The level of mortality subsequent to the late larval stage can be inferred by estimating the seasonal production of late-stage larvae on a per square metre basis and comparing this with existing estimates of the number per square metre of age 1 and 2 scallops. On the northern flank and northeast peak (areas 1 and 2 in Fig. 4) the mean abundance of late-stage sea scallop larvae (those >233 μm) ranged from ~40 to 500 individuals·m^{-2} (Table 4). Given that this range of abundance occurs for at least 21 d and that individual late-stage larvae are present for an average of 7 d before settling, a coarse estimate of the autumn production of late-stage larvae for this part of Georges Bank is 120–1500 individuals·m^{-2} (21 d × 40·m^{-2}/7, 21 d × 500·m^{-2}/7). Actual densities of sea scallops are not well estimated by the scallop drags used in fishing because drag efficiency is low and related to scallop size (Caddy 1968, 1972, 1989). From camera surveys of a large bed (274 km^2) on the northeast peak in 1970 and 1971 the density of sea scallops (ages 3 and 4) averaged ~1·m^{-2}, but reached as high as 5·m^{-2} in the centre of the bed (Caddy 1971, 1975). Using a quantitative epibenthic sled, the density of small scallops (ages 1 and 2) has recently been estimated to be ~0.7·m^{-2} on the northeast peak (Thouzeau et al. 1991). Given the much lower density of settled individuals compared with late-stage planktonic larvae, substantial mortality must occur during metamorphosis, settlement, and the early juvenile stages.

The supply of pelagic larvae can determine the structure of some intertidal invertebrate communities (Connell 1985; Gaines and Roughgarden 1985; Sutherland 1987) and is thought to limit the abundance of some marine fish (e.g. Victor 1986) and scallops (Sause et al. 1987). On Georges Bank, it seems unlikely that at present the supply of scallop larvae is limiting recruitment to the population. Larval supply may well limit scallop recruitment in other offshore areas, such as Browns Bank, where production of scallop larvae is low due to the low spawning biomass, and it appears that there is little immigration of larvae from Georges Bank. Those larvae which are produced on Browns Bank have a high probability of being lost because physical retention there is relatively low (Loder et al. 1988).

Emigration of scallop larvae from Georges Bank to the Gulf of Maine and Scotian Shelf is limited, but exchange of larvae among aggregations on Georges Bank is probable. Better quantification of the exchange of sea scallop larvae should be possible with the development of improved models of the physical circulation in the Georges Bank area.

Acknowledgements

Scott Wilson and Darlene Meade are specially thanked for their willing and able assistance in the field and the laboratory. For help with sampling, we thank P. Cranford, D. Duggan, R. Duggan, G. Harding, P. Hurley, M. Lundy, J. Muelbert, A. Orr, S. Rao, J. Reid, D. Reimer, D. Roddick, R. Semple, A. Silva, P. Vass, and the crew of the RV *Lady Hammond*. W. Smith and the National Marine Fisheries Service are thanked for obtaining plankton samples in September 1987. We thank J. Loder, G. Robert, and F. Serchuk for providing unpublished data and G. Black for help with graphics. For comments on earlier drafts of this paper, we thank S. Campana, J. Loder, G. Lough, I. McLaren, G. Newkirk, I. Perry, D. Pezzack, G. Robert, R. Scheibling, and two anonymous referees. The 1988 physical oceanographic program was funded in part by the Interdepartmental Panel on Energy, Research and Development. This work forms part of a Ph.D. thesis submitted to Dalhousie University by M. J. Tremblay.

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Ruffe, *Gymnocephalus cernuus*: Newly Introduced in North America[1]

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Pratt, D. M., W. H. Blust, and J. H. Selgeby. 1992. Ruffe, *Gymnocephalus cernuus*: newly introduced in North America. Can. J. Fish. Aquat. Sci. 49: 1616–1618.

The Eurasian ruffe, *Gymnocephalus cernuus*, was collected from the lower St. Louis River, Lake Superior's westernmost tributary, in late summer 1987. This is the first known occurrence of the ruffe in North America. The likely vector for this species was ballast water of a transoceanic vessel dumped into the international port of Duluth-Superior located on the lower end of the St. Louis River. The ruffe is increasing in abundance and expanding its range into other tributaries and nearshore areas of Lake Superior.

La grémille, *Gymnocephalus cernuus*, a été capturée pour la première fois en Amérique du Nord dans le cours inférieur de la rivière Saint-Louis, le tributaire le plus à l'ouest du lac Supérieur, vers la fin de l'été 1987. Les eaux de ballast relâchées par un navire étranger dans le port international de Duluth-Supérieur, situé à l'embouchure de la rivière Saint-Louis, est le vecteur probable de cette espèce d'origine européenne. Son abondance augmente et son aire de répartition s'étend dans d'autres tributaires et dans les zones côtières du lac Supérieur.

Received May 29, 1991
Accepted February 14, 1992
(JB058)

Reçu le 29 mai 1991
Accepté le 14 février 1992

The ruffe, *Gymnocephalus cernuus*, a Eurasian percid (Fig. 1), is found in fresh and brackish water (Lind 1977), in lakes and slow-flowing rivers in England, eastern and northern Europe, and Asia, in rivers emptying into the Arctic Ocean, and in the Caspian and Aral seas (Berg 1949; Holcik and Mihalik 1968). The ruffe has become overly abundant where introduced in Europe (P. Maitland, Easter Cringate, Stirling FK7 9QX, Scotland, U.K., pers. comm.), and its growth may be stunted (Fedorova and Vetkasov 1973). Ruffe are highly fecund (Berg 1949; Bacmeister 1977) and have been reported to be predators on fish eggs and larvae (Nikolskii 1954; Mikkola et al. 1979). European literature on the ruffe indicates that this species is of little or no value as a food or recreational fish and is frequently considered a pest or even a serious problem. Our purpose here is to document the introduction of this potentially harmful species into North America.

Thirty-one small ruffe were captured at three locations in the 4400-ha St. Louis River, Lake Superior's westernmost tributary (Fig. 2), during standard index seining in July–August 1987. The specimens collected averaged 38 mm total length. Additional ruffe were collected on September 16, 1987, when they had reached an average size of 69 mm total length. These fish were tentatively identified as ruffe and the identification was confirmed by T. M. Cavender (Curator of Fishes, the Ohio State University Museum of Zoology, 1813 North High Street, Columbus, OH 43210, USA, pers. comm.).

Other agencies that had recently sampled in the St. Louis River were notified of this newly discovered species. Ichthyoplankton collections by personnel of EA Engineering, Science, and Technology, Inc., included 66 ruffe larvae from three sites in the St. Louis River during 1986 and 101 larvae from four sites in 1987. These specimens had been misidentified as johnny darters, *Etheostoma nigrum* (J. Vondruska, EA Engineering, Science, and Technology, Inc., 221 Oak Creek Drive, Lincoln, NB 68528, USA, pers. comm.). Simon and Vondruska (1991) have recently provided a description of these ruffe larvae. In addition, a U.S. Fish and Wildlife Service (USFWS) sea lamprey control team had killed a single ruffe during 3-trifluoromethyl-4-nitrophenol (TFM) treatment of the upper St. Louis River estuary on September 1, 1987, and that specimen was preserved unidentified at Marquette, MI (J. Heinrich, USFWS, Marquette, MI 49855, USA, pers. comm.).

The likely vector for this exotic species was the ballast of a seagoing vessel. The Duluth-Superior harbor is an international grain shipping port located in the mouth of the St. Louis River (Fig. 2) that is visited by many in-ballast grain ships each year. For example, 181 transoceanic vessels entered the Duluth-Superior harbor in 1988 (D. Wright, Seaway Port Authority of Duluth, 1200 Port Terminal Drive, Box 16877, Duluth, MN 55806, USA, pers. comm.). Many of these ships previously loaded and unloaded in north European sea ports that are typically located in the lower reaches of large rivers where ruffe are common.

Great Lakes fishery agencies have been plagued for over 40 yr with the disastrous consequences of invasions by nonnative fishes. We are greatly concerned that ruffe will have negative impacts on the native fishes of the Great Lakes, of other lakes and rivers throughout North America, and on the fisheries they support.

During 1988–91 the abundance of ruffe in the St. Louis River has sharply increased and ruffe have slowly spread into southwestern Lake Superior and the mouths of the Amnicon, Brule,

[1]Contribution 798 of the National Fisheries Research Center - Great Lakes, Ann Arbor, MI 48105, USA.

FIG. 1. A 165-mm-long (total length) gravid female ruffe, *Gymnocephalus cernuus*, taken from the St. Louis River. Note the prominent preopercular spines, the long, strong dorsal spines, and the two strong anal spines. Overall body form is similar to that of a chunky yellow perch, *Perca flavescens*, while coloration resembles that of the walleye, *Stizostedion vitreum*. The spotted dorsal is marked similarly to that of a sauger, *Stizostedion canadense*.

FIG. 2. Lake Superior, and sites (stars) where ruffe, *Gymnocephalus cernuus*, have been captured.

and Iron rivers (Fig. 2) which are approximately 25, 40, and 50 km east of the St. Louis River along the south shore of Lake Superior (Wisconsin Department of Natural Resources and USFWS, unpubl. data). On August 14, 1991, an angler caught a ruffe in the Kaministiquia River at Thunder Bay, Ont., Canada (Fig. 2), and turned the specimen over to Ontario Ministry of Natural Resources (OMNR) personnel (W. MacCallum, OMNR, Thunder Bay, Ont., Canada, pers. comm.). In September and October 1991, OMNR and USFWS personnel collected six additional specimens in the Thunder Bay harbor.

Thunder Bay is about 300 km northeast of the St. Louis River and there is a hostile coastline between the two areas. It appears unlikely to us that the ruffe moved along this coastline incrementally. We suspect that the ruffe found at Thunder Bay are the result of a relocation of fish from the St. Louis River via intralake shipping (ballast) and suggest that such continued movements will negate any efforts to contain and control ruffe within Lake Superior.

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Hemolymph Ecdysone Concentration as a Function of Sexual Maturity in the Male Snow Crab (*Chionoecetes opilio*)

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Cormier, R .J., A. R. Fraser, R. F. J. Bailey, and N. Raymond. 1992. Hemolymph ecdysone concentration as a function of sexual maturity in the male snow crab (*Chionoecetes opilio*). Can. J. Fish. Aquat. Sci. 49: 1619–1623.

Ecdysone concentrations in the hemolymph of juvenile and morphometrically mature male snow crab (*Chionoecetes opilio*) were determined by radioimmunoassay. A logarithmic transformation of the allometric relationship of the dry weight of the chelae versus the carapace width was used to identify morphometric maturity. Results indicate that concentrations of ecdysone found in the hemolymph of juvenile crab are higher than those found in morphometrically mature crab, thus providing a biochemical basis for the observation that the onset of sexual maturity seems to coincide with a terminal molt.

Les concentrations de l'ecdysone dans l'hémolymphe d'individus juveniles et d'individus morphométriquement matures chez le crabe des neiges mâle (*Chionoecetes opilio*) ont été mesurées par radioimmunoessai. Une transformation logarithmique de la relation allométrique du poids sec de la pince en fonction de la largeur de la carapace a été utilisée pour l'identification de la maturité morphométriquement. Les résultats démontrent que les concentrations de l'ecdysone retrouvées dans l'hémolymphe des crabes mâles juvéniles sont plus élevées que celles retrouvées chez les crabes morphométriquement matures, apportant ainsi une argument biochimique à l'hypothèse que la mue à maturité coincide avec une mue terminale.

Received April 30, 1990
Accepted February 14, 1992
(JA557)

Reçu le 30 avril 1990
Accepté le 14 février 1992

Recent studies suggest the existence of a terminal molt once the male snow crab (*Chionoecetes opilio*) has molted to morphometric maturity (Conan and Comeau 1986). Donaldson and Johnson (1988) questioned the existence of this terminal molt. They observed molting in a small proportion of morphometrically mature males. Their findings, as well as the existence of a terminal molt, were debated in subsequent papers (Conan et al. 1988a, 1988b).

In many majid (Oxyrhyncha) crabs studied to date, the molt to morphometric maturity, or puberty, is considered terminal (Teissier 1933, 1935; Hartnoll 1963, 1965; Vernet-Cornubert 1960). Arrested growth, a consequence of this molt, has been related to a degeneration of the Y organs. Carlisle (1957) noted a reduction in size of the Y organs after the molt to puberty in *Maia squinado*. Furthermore, with *Pisa tetraodon*, Y organs cannot be observed by dissection after the onset of sexual maturity (Vernet-Cornubert 1958, 1960). Chaix et al. (1976) have demonstrated with histological observation on *Acanthonyx lunulatus* that the Y organs degenerate and completely disappear after the terminal molt. The concentration of ecdysones and the size of the Y organs also decrease after the onset of sexual maturity in the isopod *Sphaeroma serratum* (Charmantier et al. 1977; Charmantier and Trilles 1979). Vernet-Cornubert (1960) in *P. tetraodon* and Hinsch (1972) in *Libinia emarginata* observed that ablation of the eyestalks, which are the sites of secretion of a molt-inhibiting hormone (MIH), results in ecdysis for juvenile individuals but not for adults. The interpretation of these results is that adults have lost the ability to molt, probably because of a degeneration of the Y organs and a consequent reduction in the concentration of ecdysones.

The Y organs, responsible for the production of the molt hormone α-ecdysone (Blanchet 1974), play a major role in the control of the molt cycle in crustaceans (Echalier 1959; Charmantier-Daures 1975). The concentration of α-ecdysone (Adelung 1969), as well as the size of cell nuclei found in the Y organs (Girard and Maissiat 1983), varies according to the stage of the molt cycle. For example, during intermolt, the concentration of ecdysones drops dramatically to some basal level and remains there until the next ecdysis (Soumoff and Skinner 1983).

Acting as a secondary sexual characteristic (Teissier 1933; Conan and Comeau 1986), the volume of the chelae increases disproportionately to the size of the carapace during the molt to morphometric maturity. This allometric relationship is used

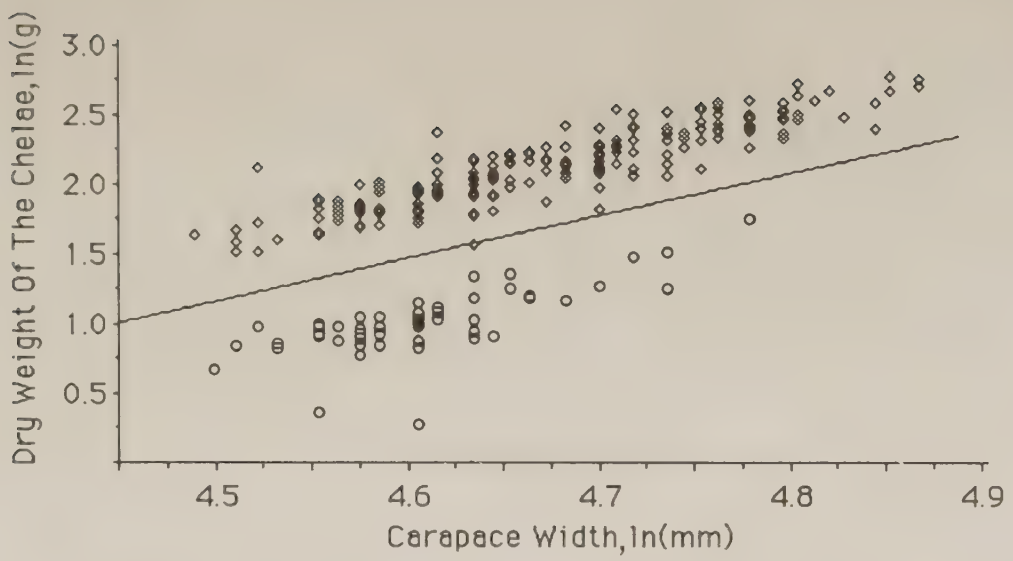

FIG. 1. Scattergram of the weight of the chelae versus the carapace width, both projected on a logarithmic scale (circles = juvenile male snow crab; diamonds = mature male snow crab).

in this paper as a tool to separate the juvenile and morphometrically mature male snow crab. Based on this classification, we have determined, by radioimmunoassay, the concentration of ecdysones in juvenile and morphometrically mature male snow crab to test the hypothesis of a terminal molt after the molt to morphometric maturity.

Material and Methods

Male snow crab were collected using commercial rectangular as well as standard conical traps during two expeditions in the baie des Chaleurs south of Chandler, Province of Québec, in May and June of 1984.

Allometric Measurements

The dry weight of the chelae rather than their linear measurements (Conan and Comeau 1986) was used in determining the allometric relationship with the carapace width. The flesh and the cuticule of the chelae were dried separately at a temperature of 60°C for a period of 24 h and weighted to the nearest milligram. The combined weight was used as the dry weight of the chelae. The overall carapace width was measured to the nearest millimetre using calipers.

The projection, on a logarithmic scale, of the scattergram showing the dry weight of the chelae versus the width of the carapace of 265 male snow crab was used to separate juvenile and mature males (Fig. 1). Radioimmunoassays were conducted on 13 juvenile and 15 mature individuals.

Determination of Molt Stage

The molt stage of each crab was determined by histological section of the dactylopod of the fifth right pereiopod. These dactylopods were fixed in Bouin solution for 24 h and subsequently dehydrated and stored in a 70% ethanol–water solution. Each paraffin-coated sample was sectioned transversally to give 5-μm sections which were stained using the method of Mallory (Gabe 1968). Only those snow crab which were considered to be intermolt (Drach and Tchernigovtzeff 1967) were selected for radioimmunoassay.

Hemolymph Preparation

Hemolymph was collected at the time of capture from the joint between he coxopod and the ischiopod using a 10-mL syringe. The samples were frozen at −80°C before being lyophilized. They were then rediluted to give hemolymph concentrations of 4×, 2×, 1×, 0.5×, and 0.25× prior to duplicate radioimmunoassay on each dilution. This provided a range of hemolymph concentrations to ensure that at least one assay sample would fall within the reliable range of detection of the radioimmunoassay.

Radioimmunoassay

Antibodies were produced using the method of Borst and O'Connor (1974). The antigen used was α-ecdysone (2β,3β,-14α,22[*R*],25-pentahydroxy-7-cholesten-6-one, 95–98%; Sigma Chemical Company Inc., St. Louis, MO). The optimum sensitivity of a competitive radioimmunoassay occurs at serum dilutions where antibodies bind 50–70% of available antigens (Skelley et al. 1973). A serum dilution of 1 in 2000 was found to bind 67% of available antigen and was used for all assays.

Assays were conducted using a method developed for steroids by R. Robinson and A. Swan (Nova Scotia Agricultural College, Truro, N.S., pers. comm.). The competitive agent in each assay was [^{3}H] α-ecdysone (New England Nuclear, Boston, MA) with a specific activity of 58.8 Ci/mmol (1 Ci = 37 GBq). A standard linear curve for α-ecdysone concentrations was established using the logit of the percentage bound counts versus the log of the known α-ecdysone serial dilution concentrations (Rodbard et al. 1968).

Cholesterol (5-cholesten-3β-ol, 99%; Sigma Chemical Company Inc., St. Louis, MO) and β-ecdysone (2β,3β,14α,-20β,22,25-hexahydroxy-7-cholesten-6-one, 95%; Sigma Chemical Company Inc., St. Louis, MO) were assayed to determine the level of affinity these molecules have for the antibodies to α-ecdysone. The construction of standard curves using these compounds permitted an evaluation of their degree of competition for the antibodies.

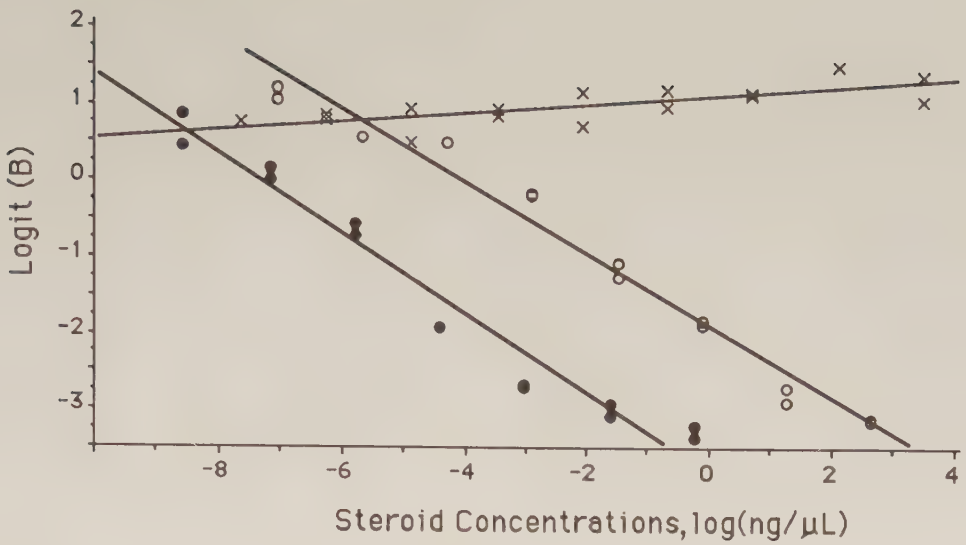

FIG. 2. Logit of the percent of [^{3}H]α-ecdysone bound to the antibodies as a function of steroid concentrations (solid circles = α-ecdysone; open circles = β-ecdysone; crosses = cholesterol) as projected on a logarithmic scale.

The mean hemolymph ecdysone concentration of each juvenile and mature group was compared using an unpaired Student *t*-test conducted on the log-transformed data.

Results

Competitive α-Ecdysone Analogues

β-Ecdysone was found to compete with the tritiated α-ecdysone for antigen binding sites in the antibodies produced. However, the concentration of this compound must be 28 times higher than that of α-ecdysone in order to compete successfully at the level of 50% [^{3}H]α-ecdysone bound (Fig. 2). Although the antigenicity of β-ecdysone is small with respect to that of α-ecdysone, it cannot be ignored. For this reason, we will use the term "ecdysones" when referring to the hormones detected in the hemolymph radioimmunoassays. However, cholesterol does not compete at all for the antibodies made toward α-ecdysone (Fig. 2).

Radioimmunoassay of Snow Crab Hemolymph

Hemolymph ecdysone concentrations in male snow crab are found to be low during intermolt and fall close to the lower limit of optimum sensitivity of the radioimmunoassay. Lyophilization of hemolymph followed by redilutions to higher hemolymph concentrations provides ecdysone concentrations falling within optimum sensitivity. Hemolymph dilutions of 1× and 2× for juveniles and 2× and 4× for mature males are found to be adequate for this radioimmunoassay.

The concentrations of ecdysones found in the hemolymph of mature and juvenile male snow crab are shown in Table 1. The average ecdysone concentration found in the hemolymph of juvenile males (9.269 ng/mL) is sigificanly higher ($\alpha = 0.01$) than the average ecdysone concentration found in the hemolymph of mature males (2.551 ng/mL).

Discussion

Morphometric measurements have been used in the past to determine maturity in snow crab (Powles 1968; Watson 1970; Conan and Comeau 1986). However, in this study, we decided to use the dry weight of the chelae rather than the linear measurements used by Conan and Comeau (1986). Using this method, a better separation of the two aggregates of points was obtained. It must be cautioned, however, that before undertaking the use of chelae weights in an allometric relationship of this type, the molt stages of the animals must be evaluated to account for the variability of cuticle thickness and muscle mass during the molt cycle (Drach 1939).

A small number of mature males were found to have ecdysone levels comparable with those in juvenile animals. Allometric relationships reflect morphometric events occurring during the onset of sexual maturity. Morphometric events may not reflect physiological events occurring within the animal which may take longer periods of time. Animals used in this study are from a commercially exploited stock. Males are found with clean carapaces, indicating that they have recently molted. Thus, the limited sample of mature individuals with higher ecdysone levels may be animals which have recently acquired their secondary sexual characteristics and still have somewhat active Y organs. Further studies would be required to determine the variation of ecdysone concentrations in relation to molt stages. In this study, histological examination of the cuticule is used to determine the intermolt stage and thus minimize the effect of molt stage on ecdysone concentration.

Teissier (1933) noted that male majids undergo a terminal molt with the onset of sexual maturity. Conan and Comeau (1986) found that mature males of *C. opilio* are seldom found in premolt stages. These findings lead them to the suggestion that males undergo a terminal molt as do females (Yoshida 1941; Rioux 1988). In our study, the level of ecdysones found in juvenile males should be considered as intermolt basal levels for their size range. Mature males would be expected to have similar basal levels if they are still actively molting. However, mature males are found to have hemolymph ecdysone concentrations approximately three times lower than their juvenile counterparts. Bearing in mind the putative relationship between ecdysone levels and molting behavior in a number of species (Adelung 1969; Charmantier et al. 1977), our findings are consistent with current thought regarding the arrest of snow crab molting with the onset of sexual maturity (Conan et al. 1988b).

Fishery recruitment is an important factor to take into consideration in population dynamics of a commercially exploited

TABLE 1. Hemolymph ecdysone levels of juvenile and mature male snow crab deterined by radioimmunoassay.

Juvenile male snow crab			Mature male snow crab		
Ecdysone (ng/mL)	Size (mm)	Chelae (g)	Ecdysone (ng/mL)	Size (mm)	Chelae (g)
1.061	94	2.369	0.820	113	8.352
2.774	105	2.513	0.957	106	8.730
4.189	95	2.475	1.223	92	4.523
6.825	100	2.419	1.247	100	6.093
8.325	107	3.347	1.381	110	8.080
10.320	99	2.538	1.457	130	15.325
10.500	103	2.579	1.510	110	8.210
11.000	102	3.061	1.532	104	8.775
11.000	94	2.305	1.547	110	8.764
13.000	101	2.753	1.579	105	17.925
13.000	100	2.822	1.775	110	9.945
13.500	107	3.318	2.304	98	6.336
15.000	99	2.311	5.245	93	8.275
			6.587	99	6.036
			9.099	106	7.761
Mean 9.269			2.551		

stock. Size limit is the primary approach used today in the management of commercial *C. opilio* stocks. This approach was originally believed to protect the smaller mature males from being exploited by the fishery, enabling them to reproduce the stock before molting to a commercial size (Watson 1970). The prospect of a terminal molt at maturity in *C. opilio* complicates a size limit concept of management because smaller mature males are unlikely to molt to a comercial size. With the existence of a terminal molt in *C. opilio*, recruitment into the fishery would depend on the proportion of juveniles of a given cohort molting to maturity at sizes greater than the established size limit (Conan and Comeau 1986; Bailey and Elner 1989; Comeau and Conan 1992).

Acknowledgements

We thank Michel Comeau of the Marine Biology Research Laboratory for offering helpful comments and suggestions as well as the external referees for their thoughtful reviews.

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Reliability of Selection Curves Obtained from Trouser Trawl or Alternate Haul Experiments

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Cadigan, N. G., and R. B. Millar. 1992. Reliability of selection curves obtained from trouser trawl or alternate haul experiments. Can. J. Fish. Aquat. Sci. 49: 1624–1632.

This study presents simulation results from a comparison of three methods for estimating selection curves from selectivity trials where the experimental codend of a trawl is fished in conjunction with a "control" (small mesh) codend. Methods 1 and 2 are based on the traditional approach of fitting selection curves to the so-called "proportion retained," the ratio of experimental codend to control codend catch, at each length class. Method 3 fits to the proportion of the total (control codend plus experimental codend) catch of each length class that is from the experimental codend. The traditional approaches exhibited considerable bias and consistently overestimated the 50% retention length and selection range. This partly explains why, in the published literature, trouser trawl experiments typically give higher estimates of the 50% retention length than covered codend experiments. Method 3 displayed little or no bias and always had lowest mean square error of the three methods. It is shown that the 50% retention length and selection range estimated by method 3 are approximately normally distributed for modest sample sizes.

La présente étude montre les résultats d'une comparaison de trois méthodes pour estimer les courbes de sélectivité à partir d'essais de sélectivité dans lesquels on utilise pour pêcher le cul-de-chalut expérimental d'un chalut en conjonction avec un cul-de-chalut « témoin » (à mailles fines). Les méthodes 1 et 2 sont basées sur l'approche classique de l'ajustement des courbes de sélectivité à ce qu'on appelle la « proportion retenue », le rapport des prises avec le cul-de-chalut expérimental à celles obtenues avec le cul-de-chalut témoin, pour chaque catégorie de longueur. Dans la méthode 3, on ajuste les courbes à la proportion des prises totales (cul-de-chalut témoin plus cul-de-chalut expérimental) de chaque catégorie de longueur provenant du cul-de-chalut expérimental. Les approches classiques ont produit des erreurs systématiques considérables et ont immanquablement surestimé la longueur de rétention à 50 % et l'intervalle de sélection. Cela explique en partie pourquoi, dans la documentation publiée, les expériences utilisant un chalut pantalon produisent généralement des estimations de la longueur de rétention à 50 % supérieures à celles des expériences utilisant un cul-de-chalut couvert. La méthode 3 a produit peu ou pas d'erreurs systématiques; il s'agit de la méthode où l'erreur quadratique moyenne était la plus faible. Nous montrons que la longueur de rétention à 50 % et l'intervalle de sélection estimés par la méthode 3 ont une distribution approximativement normale pour des tailles d'échantillons modestes.

Received August 26, 1991
Accepted February 10, 1992
(JB196)

Reçu le 26 août 1991
Accepté le 10 février 1992

Maximizing the yield per recruit of a commercial fishery requires specification of the size at which fish should become susceptible to capture by the commercial fishing gear. In striving for "optimal" trawl gear, recent studies in trawl selectivity have used codends constructed from meshes of varying shapes and sizes. Inclusion of escape windows and adjustment of the codend shape (e.g. through tensioning of the support ropes) have also been investigated. If these modifications to the codend are to be objectively assessed, then reliable statistical analysis of data from selectivity experiments is required.

Knowledge of the size selectivity of the fishing gear also enables the population length distribution of the fishery to be estimated from commercial catch data. From this the age distribution of the population could be inferred.

Some trawl selectivity studies use a small-mesh cover attached around the outside of the experimental codend. In such studies the total number of fish of each length class that enter the codend is observed because these fish are retained by either the codend or the cover. Estimation of the selection curve is therefore a straightforward application of an analysis of binary data (Pope et al. 1975; McCullagh and Nelder 1989). However, in practice it can be difficult to prevent the cover from affecting the selection performance of the codend. This difficulty is avoided by fishing the codend without a cover, but in conjunction with a "control" codend, a small-mesh codend that serves to sample (without selection) the population being fished. The trouser trawl (Russell and Edser 1926) and variants such as twin trawls and split trawls fish the two codends simultaneously. Alternate haul studies either alternate or randomize (Bergh et al. 1990) the use of the two codends throughout a study. Recent use of trouser trawls or alternate hauls has been made by Isaksen and Larsen (1988), Simpson (1989), Pikitch et al. (1990), Suuronen (1990), Suuronen et al. (1991), and Walsh et al. (1992).

Statistical analysis of trouser trawl and alternate haul data is complicated by not knowing the number of fish of each length class that enter the experimental codend. Traditional analyses are based on the procedure described by Pope et al. (1975) that calculates the "proportion retained," the ratio of experimental codend to control codend catch frequencies, for each length class. This approach implicitly assumes that it is reasonable to use the length class frequencies in the small-mesh codend catch as a substitute for the unobserved length class frequencies of

fish entering the experimental codend. An obvious deficiency is that the "proportion retained" exceeds unity whenever the catch of a particular length class is higher in the experimental codend, a common occurrence for length classes of larger fish. This study investigates the performance of two methods based on this traditional approach. The two methods differ in how they manipulate the "proportion retained" values prior to statistical fitting of the selection curve.

The third method studied is the SELECT (**S**hare **E**ach **LE**ngthclass's **C**atch **T**otal) model (Millar 1991a) as applied to trouser trawl and alternate haul data (Millar and Walsh 1992). This method fits a curve to the proportion of the total (experimental codend plus control codend) catch of each length class that is from the experimental codend, and from this curve the selection curve is then derived.

An additional complication in the analysis of trouser trawl and alternate haul data is that the small-mesh and experimental codends may have different fishing efficiencies. That is, there may be a tendency for more fish to enter one codend than the other. This occurs frequently in practice, for reasons discussed by Pope et al. (1975) and Millar (1991a). The SELECT method can incorporate unknown relative fishing efficiencies as part of the model. There is no explicitly defined way to incorporate it into the traditional methods. This consideration is discussed further in the Methods section.

Selection curves are most commonly summarized by the length at which retention probability is 0.5 (l_{50}) and by the selection range (SR = $l_{75} - l_{25}$), the difference between the lengths of 0.75 and 0.25 retention probability. The three methods were compared in terms of their accuracy and precision in estimating these two quantities. To assess the effect of sample size, selection range, and population length distribution, combinations of different levels of these three factors were varied throughout the simulations.

The SELECT method uses maximum likelihood theory to provide standard error estimates for l_{50} and SR (Millar and Walsh 1992). The effect of sample size on the distribution of the SELECT estimates was studied with particular interest in the validity of these estimated standard errors.

Methods

The logistic function is used here to model the selection curve because it is the most commonly used mathematical description of codend selectivity. Let $r(l)$ be the probability that a fish of length l, having entered the experimental codend, is retained. Then,

$$(1) \quad r(l) = \frac{\exp(a + bl)}{1 + \exp(a + bl)} .$$

In this study the three methods presented below all fit this selection curve. That is, they estimate parameters a and b in (1), from which the estimated l_{50} and SR are derived (Pope et al. 1975).

Method 1

Pope et al. (1975) presented a procedure for preliminary processing of the data. To allow for a possible difference in the fishing efficiency of the two codends, the small-mesh codend catch frequencies are scaled before calculation of the "proportion retained." The scaling factor is the ratio computed by dividing the total number of large fish (i.e. those above the effective range of selection) caught in the experimental codend by that caught in the small-mesh codend. Pope et al. (1975) did not present specific criteria concerning when to apply this scaling or on determining what lengths are above the effective range of selection of the experimental codend. In their example (haddock (*Melanogrammus aeglefinus*) retention by an 87-mm diamond-mesh codend) the ratio is calculated using fish 32 cm and longer. However, in a SELECT analysis of this data, Millar (1991a) estimated only 84% retention at 32 cm, suggesting that the scaling factor was underestimated.

Pope et al. (1975) fitted a curve to their example haddock data by eye and so were not bothered by length classes for which the "proportion retained" exceeded unity. Walsh et al. (1989) used probit analysis on their American plaice (*Hippoglossoides platessoides*) data and set "proportion retained" to 1.0 whenever it exceeded this value. Our implementation of method 1 follows this latter approach, except that instead of probit analysis (maximum likelihood fitting of normal probability ogives), we use logit analysis (maximum likelihood fitting of the logistic curve (1)). Any length classes with no fish in the small-mesh codend were discarded. The logit analysis was performed using the *glim* function in the statistical software package *Splus*.

Method 2

Simpson (1989) followed Pope's preliminary processing of the data. To determine whether the length class frequencies in the small-mesh codend should be scaled, Simpson used a chi-square test to determine if the number of fish larger than the "length at 100% retention" was significantly different between the two codends. Prior to statistical analysis, all length classes above the first length class at which a three-point moving average of the "proportion retained" value equaled or exceeded 1.0 were discarded. The value that equaled or exceeded 1.0 was set to 0.99 to accomodate the method of analysis. We followed Simpson's procedure up to this point, but whereas Simpson used nonlinear least squares to fit the logistic selection curve, we again used logit analysis. The only difference between our implementations of methods 1 and 2 is that method 2 smooths the data and discards data once the smoothed "retention probabilities" reach unity.

Remark: Strictly speaking, methods 1 and 2 are not defined when the relative catching efficiencies of the two codends are unknown. This is because the scaling factor is based on catch ratios of fish above the "length at 100% retention," which is not explicitly defined. In fact, the length of 100% retention for the logistic curve (1) is infinity.

Method 3

The SELECT method (Millar 1991a; Millar and Walsh 1992) is free of the above problems concerning "proportions" being undefined or exceeding unity and explicitly includes the possibility of unknown relative fishing efficiencies. It fits the selection curve and a split parameter p to quantify the relative fishing power of the two codends. Parameter p is the expected proportion of the total number of fish entering the two codends that enter the experimental codend. So, $p = 0.5$ corresponds to equal fishing efficiency and $p > 0.5$ to greater fishing efficiency of the experimental codend. For the logistic selection curve (1), Millar and Walsh (1992) show that the expected proportion of the total catch of length l fish that is from the experimental codend is given by

$$(2) \quad \phi(l) = \frac{p \exp(a + bl)}{(1 - p) + \exp(a + bl)} .$$

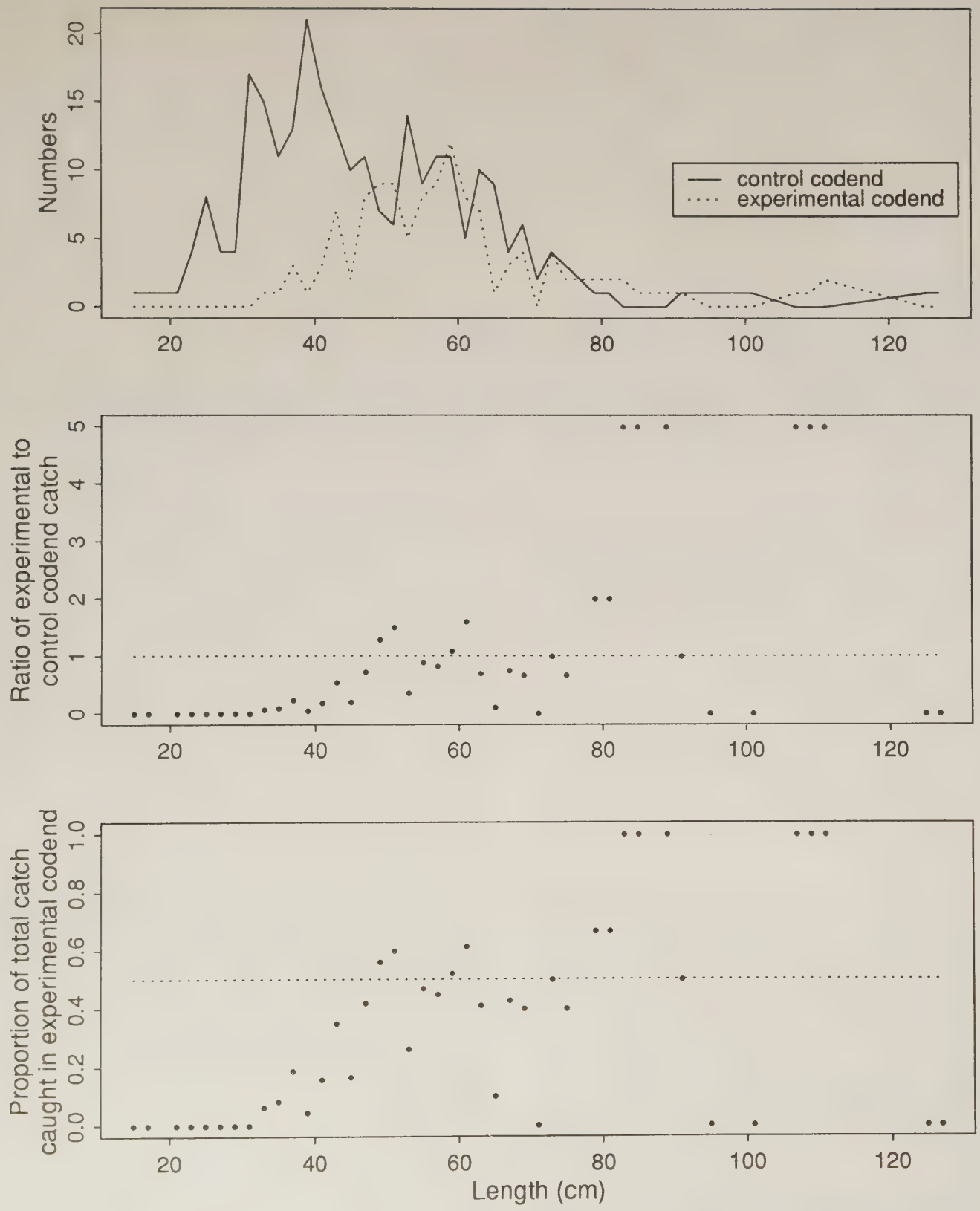

FIG. 1. Plots showing the type of data generated by the simulations: the catch frequencies in the experimental and control codends (top), the ratio of experimental to control codend catch (middle), and the proportion of the total catch taken by the experimental codend (bottom). The population length distribution of fish had mean 48 and the true selection curve had l_{50} and selection range (SR) of 45 and 9 cm, respectively. Equal fishing efficiency of the codends was assumed.

TABLE 1. (a) Biases of l_{50} estimated from the traditional methods (methods 1 and 2) and the two-parameter SELECT model (method 3) and (b) the root mean square errors of the l_{50} estimates. Three levels of true selection range (SR), mean population length (μ), and sample size (N) have been used. True l_{50} is 45 cm. The experimental and control codends have equal fishing efficiency.

		N = 250 SR			N = 500 SR			N = 1000 SR		
Method	μ	7	9	11	7	9	11	7	9	11
					(a) Biases					
1	43	10.2	10.3	11.3	7.2	6.7	7.2	4.8	4.9	4.7
	48	10.8	11.3	10.7	6.6	6.3	6.5	4.3	4.0	4.1
	53	8.6	8.4	7.8	4.8	4.5	3.4	2.3	2.6	2.2
2	43	1.8	2.3	2.6	1.5	1.4	1.9	0.8	1.1	1.1
	48	3.4	2.2	2.2	1.2	1.3	1.3	0.8	0.8	1.0
	53	1.6	1.2	1.6	1.1	1.0	1.2	0.4	0.8	0.7
3	43	−0.2	0.1	0.3	0.0	0.1	0.4	−0.1	0.1	0.0
	48	−0.1	0.5	0.2	0.1	−0.0	0.0	−0.1	−0.1	0.1
	53	−0.1	−0.2	−0.0	0.1	−0.0	−0.1	−0.2	−0.0	−0.1
					(b) Root mean square errors					
1	43	12.1	12.3	13.5	8.0	7.6	8.2	5.2	5.3	5.2
	48	17.0	13.6	13.5	7.3	7.4	7.7	4.9	4.5	4.8
	53	14.0	17.8	18.0	6.3	5.9	5.1	3.0	3.6	3.4
2	43	4.4	5.5	6.1	2.9	3.0	3.6	1.8	2.1	2.2
	48	25.1	4.7	5.7	2.4	3.2	2.8	1.9	1.7	2.0
	53	3.6	3.5	4.5	2.3	2.4	2.6	1.4	1.9	1.7
3	43	2.5	2.8	3.4	1.6	1.9	2.0	1.1	1.3	1.2
	48	2.2	2.7	3.4	1.4	1.9	2.1	1.1	1.2	1.4
	53	2.3	2.9	3.4	1.5	1.8	2.0	1.0	1.3	1.5

Parameters a, b, and p can be estimated using the *nlmin* function in *Splus* or with the FORTRAN program of Millar and Cadigan (1991).

Millar and Walsh (1992) use the likelihood ratio test for the hypothesis of equal fishing efficiency, H_0: $p = 0.5$. This involves fitting (2) with p fixed at 0.5 and comparing that ft with the fit obtained with p estimated. Twice the difference in the log-likelihoods from the three-parameter (estimated p) fit and the two-parameter (p fixed at 0.5) fit is approximately distributed as a chi-square random variable with one degree of freedom, under the null hypothesis of $p = 0.5$

Method 3 requires more attention to fit than method 1 or 2 because *nlmin* is a general function minimizer whereas *glim* is designed specifically for fitting generalized linear models, which include logit analysis. Initial guesses of a, b, and p (when estimated) are required as starting values by *nlmin*. In practice, good starting values for a and b can be calculated from rough guesses of l_{50} and SR, and 0.5 is an adequate initial guess for p. Variability in starting values was included in the simulations by using the values obtained by multiplying each of the true parameter values by a uniform (0.9, 1.1) random variable.

Simulations

The first step of each replication was to generate values from the length distribution of the population being fished. We chose this to be a lognormal distribution because between the lognormal, normal, and gamma distributions, it was the one providing the best fit to 27 yr of data (1963–89) from spring surveys of cod in NAFO region 3L (northern Grand Bank of Newfoundland). Lengths were generated as exp (Z) where Z was a normal random variable with mean ζ and standard deviation σ.

Parameters used in the simulation were loosely based on the offshore fishery in this region, which has a regulated minimum mesh size of 130 mm. The true l_{50} of the experimental codend was fixed at 45 cm and σ was fixed at 1/3. The factors varied were (1) expected sample size, N = 250, 500, or 1000, (2) selection range of the selection curve, SR = 7, 9, or 11 cm, and (3) mean of logged population length distribution, ζ = 3.705645, 3.815645, or 3.914736, corresponding to mean population length μ = 43, 48, or 53 cm, respectively. In the equal fishing efficiency case ($p = 0.5$), simulations were performed for all 27 combinations of the different levels of these factors. Three values of p (0.4, 0.5, and 0.6) were used when simulating the methods in the case of unknown relative fishing efficiencies, making a total of 81 combinations.

Each simulation performed 200 replications. The steps in creating data for each replication were (1) generate a Poisson (N) number, n, corresponding to the actual number of fish entering the two codends, (2) generate n random numbers from the lognormal population length distribution, (3) split the n fish into two groups, corresponding to entry into the experimental or control codend (a Bernoulli random variable with probability of success equal to p is used to determine the destination of each fish; here, success corresponds to entry into the experimental codend), (4) for each fish entering the experimental codend, generate a Bernoulli random variable to determine whether the fish escapes or is retained (the probability of success is $r(l)$ from equation (1), where success corresponds to retention), and

TABLE 2. (a) Biases of SR estimated from the traditional methods (methods 1 and 2) and the two-parameter SELECT model (method 3) and (b) the root mean square errors of the SR estimates. Three levels of true selection range (SR), mean population length (μ), and sample size (N) have been used. True l_{50} is 45 cm. The experimental and control codends have equal fishing efficiency.

		N = 250 SR			N = 500 SR			N = 1000 SR		
Method	μ	7	9	11	7	9	11	7	9	11
					(a) Biases					
1	43	21.8	22.9	26.1	16.5	15.9	16.9	12.1	12.2	12.2
	48	37.3	37.0	39.1	23.2	24.3	26.1	17.3	17.1	17.5
	53	59.4	69.9	72.3	36.9	38.5	36.2	25.0	26.1	27.0
2	43	2.1	3.4	3.5	2.1	1.8	2.6	1.4	1.6	2.0
	48	10.5	3.0	3.8	1.8	2.2	2.3	1.7	1.8	1.8
	53	2.4	1.4	2.9	1.8	1.8	2.2	1.3	2.1	1.8
3	43	−0.7	−0.0	0.2	−0.2	−0.0	0.2	−0.1	−0.0	0.0
	48	−0.5	0.2	0.3	−0.2	−0.3	0.1	−0.1	−0.0	−0.1
	53	−0.7	−0.4	0.4	−0.1	−0.2	−0.3	−0.1	−0.0	0.0
					(b) Root mean square errors					
1	43	25.4	26.9	31.0	17.8	17.3	18.8	12.6	12.9	13.2
	48	75.9	43.5	46.9	24.8	26.7	28.6	18.2	17.9	18.7
	53	79.8	109.0	94.1	40.0	41.8	40.1	26.1	27.6	28.8
2	43	7.4	9.3	9.8	5.0	4.4	6.0	2.8	3.3	3.8
	48	117.2	9.4	11.1	4.4	7.7	4.7	4.1	3.7	3.7
	53	11.8	5.2	9.2	4.2	4.9	5.1	3.1	4.5	3.7
3	43	2.9	3.6	4.8	1.8	2.4	2.7	1.3	1.5	1.7
	48	2.9	3.5	5.2	1.8	2.5	3.0	1.3	1.7	2.0
	53	3.2	4.2	6.6	1.9	2.5	3.1	1.3	1.8	2.2

(5) group the lengths of fish caught by each codend into 2-cm length classes.

Note that what we call the sample size (N) is the expected number of fish entering the trawl, and the actual number (n) entering is a Poisson random variable with parameter N. Also, because of escapement through the experimental codend, the number of fish caught will be less than n. For example, in the simulation with mean population length μ = 43 cm and SR = 7 cm, the experimental codend retains an expected 39.6% of fish that enter. If p = 0.5, then the expected number of fish caught by the trouser trawl is 69.8% of n (50% from the control codend and 19.8% from the experimental codend).

A typical example of the type of simulated data is presented in Fig. 1. Here, the mean population length μ = 48 cm and SR = 9 cm. The number of fish entering the trawl was set to 500. The expected split of fish into the two codends was assumed to be 50:50, corresponding to equal fishing efficiencies, and in this example resulted in 258 fish entering the control and 242 entering the experimental codend. The length class frequencies of fish caught by both codends are plotted in the upper portion of Fig. 1. As expected, the control codend caught more small fish than the experimental codend and both caught approximately equal numbers of large fish. The experimental codend retained 120 of the 242 fish entering it. A plot of "proportion retained" versus length is presented in the middle portion of the figure. Note that the arbitrary value of 5 is substituted for the "proportion retained" whenever it exceeds this value. A similar plot of the proportion of total catch taken in the experimental codend is presented in the bottom portion of the figure. Being true proportions, these vaues never exceed 1.

Results

Relative Fishing Efficiency of the Codends Equal

Tables 1 and 2 give the simulation results for when the split of fish into the two codends is equal. Method 3 (two-parameter SELECT) gives l_{50} and SR estimates with smaller bias and root mean square error (rmse) than method 2, and this is true over all combinations of the three factors (sample size, selection range, and population length distribution). Similarly, with just one exception, method 2 does better than method 1. The exception (N = 250, μ = 48, and SR = 7) is due to a single spurious fit in one of the 200 replications. It is possible that, for this replication, function *glim* converged to a false maximum of the likelihood function, but we did not investigate this.

Method 3 shows no obvious systematic bias in estimation of l_{50} and just a slight amount of negative bias in estimation of SR when the true SR is small. Methods 1 and 2 display considerable positive bias in the estimation of both l_{50} and SR. The biases of method 1 are extreme, even for sample size of 1000.

Other features suggested by Tables 1 and 2 are biases and rmse's decrease with increasing sample size, bias of l_{50} decreases with increasing μ for methods 1 and 2, bias and rmse of SR increase with increasing μ for method 1, rmse of SR increases with increasing μ for method 3, and rmse's of l_{50} and SR increase with increasing SR for method 3.

Relative Fishing Efficiency of the Codends Unknown

Methods 1 and 2 were implemented in the unknown relative fishing efficiency simulations by specifying fish above the 99%

TABLE 3. (a) Biases of l_{50} estimated by the three-parameter SELECT model (method 3) and (b) the root mean square errors of the l_{50} estimates. Three levels of true selection range (SR), mean population length (μ), sample size (N), and relative fishing efficiency of the experimental codend (p) have been used.

		N = 250 SR			N = 500 SR			N = 1000 SR		
Method	μ	7	9	11	7	9	11	7	9	11
					(a) Biases					
0.4	43	0.4	0.1	1.4	0.2	0.3	0.3	−0.1	0.1	0.2
	48	0.3	0.5	0.9	−0.1	−0.1	0.8	0.1	0.1	0.3
	53	0.3	0.3	1.6	−0.0	0.1	0.4	0.2	−0.3	0.3
0.5	43	0.8	1.2	1.1	−0.1	0.6	1.4	−0.1	0.2	−0.1
	48	0.8	1.3	0.9	0.2	−0.1	0.1	−0.0	−0.0	0.2
	53	0.8	0.0	0.4	0.1	0.0	0.5	−0.1	0.1	0.1
0.6	43	0.2	0.6	0.9	−0.0	0.3	0.4	−0.0	0.1	0.4
	48	0.8	0.5	1.2	0.2	0.2	0.2	0.1	0.1	0.2
	53	0.5	0.8	1.6	0.3	0.5	−0.1	0.0	0.0	0.1
					(b) Root mean square errors					
0.4	43	4.3	5.1	12.1	2.5	3.3	4.3	1.7	2.3	2.8
	48	3.9	5.1	10.0	2.0	2.8	11.7	1.3	2.0	2.5
	53	3.9	4.2	12.7	2.4	3.0	3.5	1.6	1.8	2.5
0.5	43	8.5	10.0	13.2	2.2	3.7	9.9	1.5	2.0	2.3
	48	9.5	5.2	7.2	1.9	2.6	3.3	1.5	1.9	2.4
	53	9.2	3.8	5.4	2.0	2.9	3.4	1.3	1.8	2.4
0.6	43	3.4	4.8	6.2	2.7	3.2	3.6	1.5	2.0	2.5
	48	5.8	4.5	9.1	2.0	2.6	4.1	1.4	1.9	2.1
	53	9.7	11.2	14.8	2.0	3.0	2.8	1.3	1.7	2.2

retention length of the experimental codend to be above its effective range of selection. These fish were used to calculate the scaling factor estimate of relative fishing efficiency. The choice of 99% retention length as the smallest length used in calculation of the scaling factor was totally arbitrary; in practice the decision will depend on subjective knowledge and the number of large fish caught. When many large fish are caught, this length could be higher, but with few large fish it would have to be smaller to ensure that a reasonable number of fish were used in calculating the scaling factor.

Because the simulations are unable to emulate practical application of methods 1 and 2, the simulation results obtained for these two methods are of limited value and are therefore omitted from this presentation, except to say that the relative performance of the three methods was the same as in the equal fishing efficiency case above. A different choice of the lengths used for the scaling factor would make a difference in the performance of methods 1 and 2, but it is very unlikely to make a difference in the performance rankings of the three methods (see Discussion section).

Tables 3 and 4 give the bias and rmse of method 3 (three-parameter SELECT) estimates of l_{50} and SR. In addition to varying sample size, selection range, and population length distribution, three values of the split proportion (0.4, 0.5, and 0.6) were used. The rmse's are in general higher here than for the equal fishing efficiency fits from the two-parameter SELECT model. Table 3b contains some noticeably large rmse values for the estimated l_{50}'s. In all cases, these were due to a few replications in which l_{50} was grossly overestimated. For example, in the simulation with N = 500, SR = 11, μ = 48, and p = 0.4, the rmse value of 11.7 is largely due to one estimated l_{50} of over 200 cm. This may be due to poor starting values. Methods 1 and 2 exhibited these unusual values more frequently than did method 3. A smaller length for calculating the scaling factor would have provided more data for calculating the scaling ratio and so may have reduced the occurrence of these values in methods 1 and 2.

Some other features of the three-parameter SELECT model suggested by Tables 3 and 4 are biases and rmse's decrease with increasing sample size, l_{50} tended to be slightly overestimated and SR slightly underestimated, and rmse's of l_{50} and SR increased with increasing SR.

Table 5 gives the observed power of the likelihood ratio test of equal fishing efficiency H_0:p = 0.5, tested at the 5% level of significance. Over the 27 sets of simulations conducted under the null hypothesis, the overall rejection rate was 5.2%, indicating that the actual significance level of the likelihood ratio test is very close to the desired 5%.

The alternative p = 0.6 had more power to reject the null hypothesis than the alternative p = 0.4. Under both alternatives the power consistently increased with increasing μ and decreasing SR. This is to be expected because large μ and small SR are the two combinations providing the most large fish above the "effective" range of selection of the large-mesh codend. Even though the SELECT method does not explicitly determine a length above which to estimate relative fishing efficiency, it is those fish less subject to the selective action of the experimental codend that provide the most information about relative fishing efficiencies.

TABLE 4. (a) Biases of SR estimated by the three-parameter SELECT model and (b) the root mean square errors of the SR estimates. Three levels of true selection range (SR), mean population length (μ), sample size (N), and relative fishing efficiency of the experimental codend (p) have been used.

		$N = 250$ SR			$N = 500$ SR			$N = 1000$ SR		
Method	μ	7	9	11	7	9	11	7	9	11
				(a) Biases						
0.4	43	−0.7	−0.7	−0.4	−0.2	−0.1	−0.2	−0.2	−0.1	0.1
	48	−0.4	−0.4	−0.7	−0.5	−0.5	−0.2	0.1	−0.0	0.0
	53	−0.7	−0.7	0.3	−0.5	−0.0	0.1	−0.1	−0.4	−0.1
0.5	43	−0.7	−0.2	−0.6	−0.3	0.1	0.3	−0.1	−0.0	−0.2
	48	−0.5	0.4	−0.1	−0.2	−0.5	−0.1	−0.1	−0.0	−0.1
	53	−0.5	−0.6	0.2	−0.2	−0.4	−0.0	−0.0	−0.0	0.1
0.6	43	−0.5	−0.1	0.1	−0.3	−0.1	−0.3	−0.1	−0.1	0.1
	48	−0.2	−0.2	−0.4	−0.1	−0.1	0.2	−0.1	−0.1	0.0
	53	−0.5	−0.4	0.5	−0.1	0.3	−0.3	−0.2	−0.1	−0.1
				(b) Root mean square errors						
0.4	43	3.7	4.4	5.6	2.3	2.9	3.5	1.5	2.2	2.4
	48	3.7	4.9	5.9	2.1	3.0	4.4	1.5	2.0	2.4
	53	4.1	4.9	7.8	2.7	3.4	4.1	1.7	2.0	2.6
0.5	43	3.4	4.0	5.2	2.0	2.9	3.7	1.4	1.8	2.0
	48	3.9	4.4	6.2	2.0	2.5	3.4	1.5	2.0	2.4
	53	4.7	4.0	6.6	2.1	3.0	3.8	1.4	2.0	2.7
0.6	43	3.0	3.4	4.8	2.1	2.5	2.9	1.3	1.6	2.1
	48	4.0	4.5	4.9	2.1	2.4	3.6	1.3	1.8	2.2
	53	4.2	5.4	6.5	2.2	3.3	3.4	1.4	1.9	2.5

TABLE 5. Observed power of the SELECT model likelihood ratio test (5% level of significance) for equal fishing efficiency H_0: $p = 0.5$. Three levels of true selection range (SR), mean population length (μ), sample size (N), and relative fishing efficiency of the experimental codend (p) have been used.

		$N = 250$ SR			$N = 500$ SR			$N = 1000$ SR		
Method	μ	7	9	11	7	9	11	7	9	11
0.4	43	0.28	0.31	0.24	0.47	0.38	0.35	0.77	0.67	0.56
	48	0.38	0.25	0.29	0.69	0.56	0.47	0.89	0.85	0.74
	53	0.41	0.34	0.31	0.76	0.63	0.52	0.94	0.92	0.82
0.5	43	0.05	0.07	0.05	0.05	0.04	0.06	0.06	0.06	0.04
	48	0.03	0.04	0.06	0.05	0.04	0.06	0.05	0.04	0.08
	53	0.09	0.08	0.04	0.03	0.05	0.05	0.05	0.03	0.07
0.6	43	0.35	0.37	0.27	0.59	0.61	0.55	0.88	0.87	0.83
	48	0.54	0.47	0.47	0.76	0.76	0.69	0.99	0.95	0.92
	53	0.60	0.54	0.51	0.89	0.80	0.82	0.99	0.97	0.98

Finite Sample Behaviour of the SELECT Method

SELECT is a maximum likelihood model, and therefore, the estimated l_{50} and SR are asymptotically unbiased with a limiting normal distribution (subject to certain regularity conditions). In other words, for sufficiently large sample sizes the estimates will be unbiased and normally distributed. It is then reasonable to make inferences about the true parameters based on the usual normal distribution theory, e.g. an approximate 95% confidence interval is given by the point estimator plus and minus two standard errors. These standard errors can be estimated using the formulae of Millar and Walsh (1992). This subsection presents the results of an investigation of the accuracy of the asymptotic approximation.

Figure 2 displays histograms for the estimated l_{50}'s and SR's from the three-parameter SELECT model with $\mu = 48$ cm and SR = 9 cm. The estimates displayed in Fig. 2 are from the simulations with split proportion $p = 0.6$. The normal curves overlaid on the histograms are those predicted by maximum likelihood theory calculated using the true values of l_{50}, SR, and p. The histograms resemble their predicted normal curves quite well, although for $N = 250$ the variability of the estimates appears to be underestimated. In particular, SR was estimated to be less than 0.5 cm on 9 of the 200 replications with $N =$

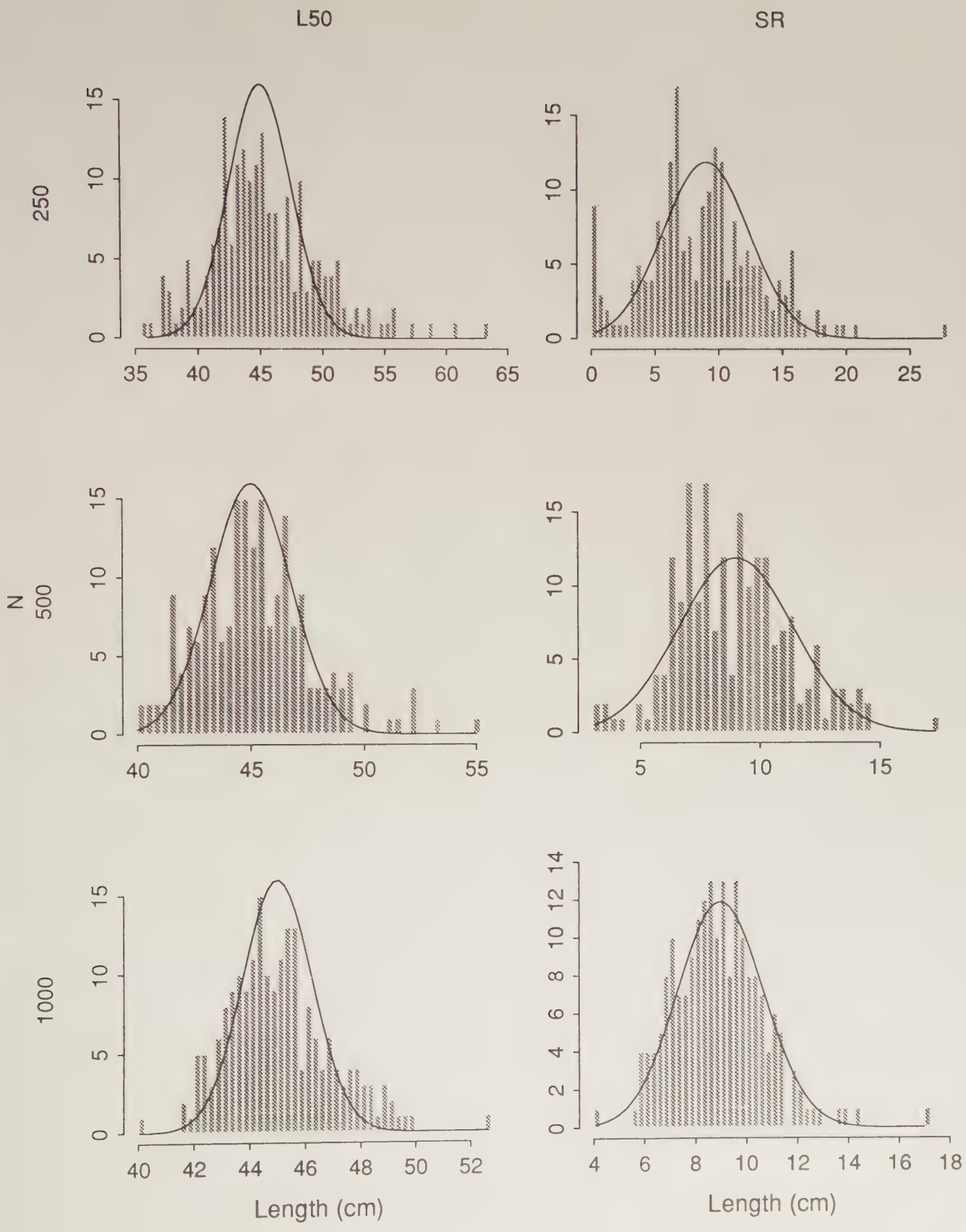

FIG. 2. Simulated and asymptotic distributions of l_{50} and selection range (SR) from the three-parameter SELECT model assuming unequal fishing efficiencies of the two codends ($p = 0.6$). The population length distribution of fish had mean 45 and the true selection curve had l_{50} and SR of 45 and 9 cm, respectively.

250. When $p = 0.5$ the approximations look better but are worse when $p = 0.4$. These results are not presented.

Discussion

It has been noted by Pope et al. (1975), Isaksen and Larsen (1988), and Simpson (1989) that covered codend studies tend to give estimates of l_{50} that are lower than those obtained by trouser trawl and alternate haul studies (using the traditional analyses), and this is usually attributed to masking by the codend cover. Our study shows that it could in part also be due to the method of analysis. Here we used logistic analysis to fit the selection curve and so our traditional analyses (methods 1 and 2) are not exactly the same as those performed by the aforementioned authors. However, it is our belief that it is the use of the "proportion retained" values that corrupts these analyses, and that even selection curves fitted by eye will be susceptible to these biases.

When the "proportion retained" exceeds unity for any length class, then that datum must be discarded, or the "proportion retained" set to unity. Values of "proportion retained" less than unity are considered valid, yet it is possibly these values that affect the analysis most. For example, consider a length class of large fish and suppose that 10 of these fish enter the gear with six entering the small mesh codend and four entering the experimental codend. The "proportion retained" is 2/3, yet the retention probability for these large fish is very close to 100%. This length class biases the analysis because the model is forced to fit a lower retention probability to this length class than it should. The simulations demonstrated the consequences: overestimated and unreliable estimates of l_{50} and SR. Method 2 performed substantially better than model 1 because it discarded most of the data for large fish.

The simulations were restricted by practicality to varying a small number of factors and assumed a logistic selection curve. Because it is the fitting to "proportion retained" values that compromises methods 1 and 2, we feel that the simulations' performance ranking of the three methods will hold in general. This includes selectivity analyses where, instead of the logistic, a different selection curve is fitted. Possibilities include normal probability ogives, Gompertz curves, and the general family of asymmetric curves used by Millar (1991b) and Suuronen et al. (1991).

In both the two-parameter and three-parameter SELECT fits (method 3) the rmse of the estimated l_{50} and SR increased as the true SR increased. This is because increasing SR corresponds to a flatter selection curve, and the flatter the selection curve the less the retention probabilities change with change in length. That is, when the true SR is very large, quite different lengths can give very similar retention probabilities. It follows that the length corresponding to a specified retention probability will be difficult to estimate. This effect of increasing SR applies to methods 1 and 2 also. It is not as evident in these methods, probably due to the large amount of variability in the estimates.

The SELECT method provides the best estimator of l_{50} and SR and the estimates are close to unbiased except when the sample size is very small. An advantage of the SELECT method is that approximate standard errors for the estimated selection lengths can be computed using standard statistical theory. For the simulation performed here the approximations were good when the expected number of fish entering the trawl exceeded 500. For $N = 250$ the approximations were less tenable due to a relatively large number of very small estimated SR's. Another advantage of the SELECT method is that, by using prior guesses of the model parameters (a, b, and p if fitted), or a range of guesses, these standard errors can be calculated before the selectiviy trial and therefore can be used in experimental design. This includes determination of the sample size requirement for a desired level of precision. The effect of altering length class width could similarly be investigated.

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Bicarbonate as a Potential Confounding Factor in Cladoceran Toxicity Assessments of Pore Water from Contaminated Sediments

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Hoke, R. A., W. R. Gala, J. B. Drake, J. P. Giesy, and S. Flegler. 1992. Bicarbonate as a potential confounding factor in cladoceran toxicity assessments of pore water from contaminated sediments. Can. J. Fish. Aquat. Sci. 49: 1633–1640.

Elevated alkalinity values measured in sediment pore water samples from the Grand Calumet River – Indiana Harbor Canal, an International Joint Commission Area of Concern (AOC), caused concern over the potential effects of alkalinity on cladoceran test responses. Forty-eight-hour tests with $NaHCO_3$ and NaCl as reference toxicants indicated that $NaHCO_3$ toxicity to *Daphnia magna* and *Ceriodaphnia dubia* was due to both Na^+ and HCO_3^-, although greater HCO_3^- toxicity was observed to *D. magna*. Theoretical HCO_3^- concentrations in sediment pore waters from the AOC demonstrated sufficient HCO_3^- in several samples to produce acute toxicity, based on 48-h LC50s from the reference toxicant tests. X-ray microanalysis was conducted to qualitatively assess the effects on internal Cl^- levels of *D. magna* after exposure to $NaHCO_3$, to an AOC sediment pore water sample, and to NaSCN, a metabolic inhibitor of Cl^- uptake. Based on these results the proposed mechanism of HCO_3^- toxicity to *D. magna* is the inhibition of the active uptake of Cl^- from water. We suggest that pore water alkalinity should be considered when interpreting the results of sediment pore water and effluent toxicity tests with *D. magna*, other cladocerans, and, perhaps, other invertebrates and fish.

Les valeurs élevées de l'alcalinité obtenues d'échantillons d'eau interstitielle de sédiments prélevés dans la rivière Grand Calumet et le canal du port d'Indiana, un secteur préoccupant de la Commission mixte internationale, ont soulevés des soucis quant aux incidences potentielles de l'alcalinité sur les réactions de cladocères en laboratoire. Des tests de 48 h utilisant du $NaHCO_3$ et du NaCl comme toxiques de référence ont révélé que Na^+ et HCO_3^- étaient à la source de la toxicité du $NaHCO_3$ pour *Daphnia magna* et *Ceriodaphnia dubia*, bien que *D. magna* semblait plus sensible à l'incidence toxique du HCO_3^-. Les concentrations théoriques de HCO_3^- dans l'eau interstitielle des sédiments prélevés dans ce secteur préoccupant ont révélé que plusieurs contenaient des teneurs en HCO_3^- suffisantes pour produire une toxicité aiguë, d'après les résultats des analyses de la CL50 après 48 h des toxiques de référence. À l'aide d'une microanalyse par radiographie, on a effectué une évaluation qualitative des incidences sur les teneurs internes de Cl^- chez *D. magna* de l'exposition au $NaHCO_3$, à un échantillon d'eau interstitielle de sédiments prélevés dans le secteur préoccupant et au NaSCN, un inhibiteur métabolique de la captation du Cl^-. D'après les résultats obtenus, on formule l'hypothès que le HCO_3^- est toxique pour *D. magna* parce qu'il inhibe la captation active du Cl^- en solution dans l'eau. On propose aussi que l'on tienne compte de l'alcalinité de l'eau interstitielle lors de l'interprétation des résultats d'analyses de l'eau interstitielle de sédiments et de la toxicité des effluents effectuées à l'aide de *D. magna*, d'autres espèces de cladocères et peut-être d'autres invertébrés et poissons.

Received March 5, 1991
Accepted February 26, 1992
(JA928)

Reçu le 5 mars 1991
Accepté le 26 février 1992

Toxicity assessment of contaminated sediments has become de rigueur in planning for remedial actions at International Joint Commission (IJC) Great Lakes "Areas of Concern" (AOC) (Hileman 1988) and at many U.S. EPA Superfund sites (Warren–Hicks et al. 1989). Sediments contaminated by a wide array of metals and organic chemicals are an acknowledged legacy of the explosive post-World War II growth in the chemical industry and the continued reliance on fossil fuels for energy production. Approaches traditionally used to evaluate contaminated sediments include (1) chemical analyses for total sediment concentrations of compounds or elements of concern, (2) toxicity tests of bulk sediments or aqueous extracts of sediments using benthic and upper water column test species, respectively, and (3) field surveys of in situ effects such as changes in benthic macroinvertebrate community structure or the occurrence of histopathological lesions in benthic fish species (for reviews, see Giesy and Hoke 1989; Chapman et al. 1987). In the present study, we focus on the potential implications of, and one of the problems involved in, the use of pore water (interstitial water) toxicity tests with cladocerans as surrogates for bulk sediment tests with benthic invertebrates.

Pore water from contaminated sediment is used as an exposure medium for toxicity tests (Giesy and Hoke 1989). Comparisons have been made of the results of tests which evaluated the toxicity to *Hyalella azteca* and *Lumbriculus variegatus* of

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bulk sediment, pore water, and elutriate from the same samples (Ankley et al. 1991). These comparisons and the results of other studies (Nebeker et al. 1984; Knezovich and Harrison 1988; van de Guchte and Maas-Diepeveen 1988) suggest that pore water exposures with benthic species are good surrogates for bulk sediment exposures.

The underlying assumption in the use of pore water exposures as a surrogate for bulk sediment exposures is that organisms receive most of their exposure to toxic substances in sediments through contact with the pore water (Nebeker et al. 1984; Ankley et al. 1991). The relationship between pore water and bulk sediment exposures has not been clearly established (Cairns et al. 1984; Nebeker et al. 1984), and many physicochemical processes can be expected to affect the partitioning of metals and xenobiotics from the solid phase into the pore water (Sinex et al. 1980; Knezovich et al. 1987; U.S. EPA 1989; Di Toro et al. 1990). Additional assumptions inherent in the use of pore water as a test medium are that basic chemical properties of pore water, such as major ion concentrations and/or ratios, are within the physiologically tolerated ranges of the test species and that the pore water extraction technique does not alter the pore water chemistry. Recent reviews of the advantages and disadvantages associated with various methods of pore water preparation can be found in Adams (1991) and Schults et al. (1992).

During an assessment of the toxicity of sediments at the Grand Calumet River – Indiana Harbor Canal (GCR–IHC) IJC AOC, several observations caused concern over the effects of chemical properties of the pore waters on the results of toxicity tests with cladocerans. Elevated pore water alkalinity values (Table 1), gas bubble formation, white crystalline residue on the inside of test chambers, and increases in pore water pH in test vessels during cladoceran toxicity tests led to the hypothesis that the test results might be affected by the altered character of the carbonate–bicarbonate buffer system in the pore waters. At the time the investigation was begun, no information existed in the literature concerning the toxicity of bicarbonate ion (HCO_3^-) to cladocerans, although some information was available on the toxicity of CO_2 (Mount and Anderson–Carnahan 1988). It also was known that CO_2 could be used to anesthetize zooplankton prior to preservation (Gannon and Gannon 1975). Limited information also was available on the toxicity of HCO_3^- to fish (Beatty 1959; Mitchum 1960).

This investigation was conducted to examine the toxicity of HCO_3^- to *Daphnia magna* and *Ceriodaphnia dubia*. The specific objectives of the study were (1) to determine the effects of HCO_3^- concentrations on *D. magna* and *C. dubia* using $NaHCO_3$ and NaCl as reference toxicants, (2) to compare calculated concentrations of HCO_3^- in sediment pore waters from the AOC study area with concentrations shown to cause adverse effects on *D. magna* and *C. dubia* in laboratory exposures, and, if HCO_3^--induced toxicity was observed, (3) to propose potential mechanisms for the observed HCO_3^--induced effects.

X-ray dispersive microanalysis previously has been used to study the role of various invertebrate tissues in metal storage and the effects of different environmental levels of metals on metal localization in invertebrates (Ballan-Dufrancais et al. 1985; Krantzberg and Stokes 1990). Investigations also have been conducted on diffusible ions in invertebrate osmoregulatory systems (Marshall and Wright 1973; Roomans 1988a) and in bulk specimens of skeletal muscle (Zierold and Schafer 1978). Because of its previous use in the examination of physiological levels of diffusible ions, X-ray dispersive microanalysis was chosen as the simplest and most direct method for monitoring the potential effects of HCO_3^- on the relative concentrations of several physiologically important elements in *D. magna*.

Materials and Methods

Toxicity of Na^+ and HCO_3^-

Forty-eight-hour acute tests (U.S. EPA 1985) were conducted with Baker reagent-grade $NaHCO_3$ and NaCl to establish the toxicity of HCO_3^- and Na^+ to *D. magna* and *C. dubia*. These exposures were initiated with <24-h-old neonates from laboratory cultures of each species and also with 6- or 7-d-old subadult *D. magna*. Six- or 7-d-old *D. magna* were initially necessary to provide specimens of sufficient size for manipulation in the preparation procedures for the X-ray microanalysis and were specifically selected for further X-ray microanalysis because of their larger size. Test organisms were not fed and the temperature and photoperiod were maintained

TABLE 1. Results of routine chemical analyses of sediment pore waters from the Grand Calumet River – Indiana Harbor Canal IJC AOC tested in 48-h acute assays with *D. magna* and *C. dubia*. pH, conductivity, hardness, and alkalinity were measured on the 100% pore water exposure treatment for each location. Free CO_2 and HCO_3^- concentrations in pore water were calculated from the measured pH, alkalinity, and temperature (25°C).

	Measured value				Calculated value	
Location	pH	Conductivity (μmho/cm^2)	Hardness (mg/L, $CaCO_3$)	Alkalinity (mequiv/L)	CO_2 (free) (mmol/L)	HCO_3^- (mmol/L)
UG-1	8.00	2570	640	13.2	0.3	13.1
UG-2	8.30	423	160	1.2	0.0	1.2
UG-3	7.70	3420	460	5.2	0.2	5.2
UG-4	8.50	830	370	7.4	0.1	7.2
UG-6	8.00	2920	2340	5.8	0.1	5.8
UG-7	7.60	1420	1040	10.0	0.6	10.0
UG-8	7.65	4300	2160	30.0	1.5	29.9
UG-9	7.86	3650	1040	20.8	0.7	20.8
UG-10	7.06	4680	2500	3.6	0.7	3.6
UG-11	7.74	2230	640	12.4	0.5	12.3
UG-12	7.60	2100	700	22.4	1.3	21.9

TABLE 2. Toxicity to *D. magna* and *C. dubia* of Na^+ as NaCl or $NaHCO_3$ and of HCO_3^- as $NaHCO_3$.

Species	Age	Compound	48-h LC50 (mmol/L)	Mean ± SD LC50 (mmol/L)
D. magna	<24 h	NaCl	85.7	81.3 ± 6.2
D. magna[a]	<24 h	NaCl	76.9	
D. magna[b]	4th instar/ adult	NaCl	57.9	—
C. dubia	<24 h	NaCl	14.3	13.5 ± 1.2
C. dubia	<24 h	NaCl	12.6	
D. magna	<24 h	$NaHCO_3$	16.6	15.1 ± 2.2
D. magna	<24 h	$NaHCO_3$	13.5	
D. magna	6 d	$NaHCO_3$	21.2	—
D. magna	7 d	$NaHCO_3$	26.3	20.6 ± 8.1
D. magna	7 d	$NaHCO_3$	14.9	
C. dubia	<24 h	$NaHCO_3$	13.8	12.8 ± 1.5
C. dubia	<24 h	$NaHCO_3$	11.7	

[a]Mount and Anderson-Carnahan (1988).
[b]Dowden (1961).

at 25°C and 16 h light: 8 h dark, respectively, during all tests. New $NaHCO_3$ and NaCl stock solutions were prepared for each test (3000 and 8000 mg/L nominal concentrations, respectively). A 0.56 × dilution series was used to prepare dilutions of the stock solutions for testing. A minimum of five toxicant concentrations and a control were prepared for each test. Five replicates, each containing five organisms, were tested for each toxicant concentration or control. The laboratory culture/diluent water was prepared by mixing bottled Perrier® mineral water and HPLC-grade laboratory water (1:9 v/v) followed by vigorous aeration for 24 h to ensure equilibration of gases prior to culture or test use (U.S. EPA 1989). Cyanocobalamin (vitamin B_{12}) and selenium (2 μg/L each), as sodium selenate, also were added to the culture/diluent water (Lanno 1989). Control survival in toxicity tests was always greater than 90%. Forty-eight-hour LC50 values were calculated using the binomial test and are reported as nominal HCO_3^- or Na^+ concentrations in millimoles per litre (Table 2).

Calculation of Free CO_2 and HCO_3^-

A series of twenty-six 48-h acute pore water tests were performed with *D. magna* and *C. dubia* as part of the original AOC sediment toxicity assessment (Hoke et al. 1992). Data from these exposures subsequently were used to calculate theoretical equilibrium concentrations of free CO_2 and HCO_3^- in the pore waters for comparison with effects concentrations from the literature or the reference toxicant tests described above. Free CO_2 and HCO_3^- concentrations at equilibrium were calculated using temperature (25°C), pH, and alkalinity values measured in the 100% pore water exposure treatment from the cladoceran tests (Table 1). Alkalinity was measured using a Hach kit (accuracy = ±0.4 mequiv/L) and pH was measured using an Orion model 701A ionanalyzer equipped with a glass Ross combination pH probe. The following equations (Harvey 1957) were used to calculate free CO_2 and HCO_3^- concentrations, respectively:

$$(1)\quad x = \alpha\beta$$

where

$$\beta = \frac{H^2}{K_1(H + 2K_2)}$$

and

$$(2)\quad y = \frac{2H}{H + 2K_2}$$

where

$H = [H^+]$

$\alpha = [HCO_3^-] + 2[CO_3^-]$ = measured alkalinity

$x = [H_2CO_3]$, including free CO_2

$y = [HCO_3^-]$

$$K_1 = \frac{[HCO_3^-][H^+]}{[H_2CO_3]}$$

$$K_2 = \frac{[CO_3^{2-}][H^+]}{[HCO_3^-]}.$$

X-Ray Dispersive Microanalysis

To investigate the effects of HCO_3^- on the internal ion balance (Na^+, Si^{4+}, Cl^-, Ca^{2+}) of *D. magna*, several 48-h tests in which $NaHCO_3$ was used as a toxicant were initiated with 6-, or 7-d-old subadult *D. magna*. Three experiments were conducted with $NaHCO_3$. Additional experiments were conducted with pore water collected from one of the original AOC sediment samples (Location UG-9) due to its high alkalinity and calculated HCO_3^- concentration, and with NaSCN, a metabolic inhibitor of Cl^- uptake (Epstein et al. 1973). Live *D. magna* were removed from the tests for subsequent X-ray analysis after 16–20 h of exposure to $NaHCO_3$ or Location UG-9 pore water. Organisms were removed for analysis after 2 h of exposure to NaSCN. Forty-eight-hour exposures to the highest concentrations of either $NaHCO_3$ or pore water were lethal to *D. magna*. Organisms to be analyzed by X-ray microanalysis were exposed for a shorter period of time because the variable of interest was change in elemental peak to background (*P/B*) ratios in living organisms as a result of exposure to $NaHCO_3$, pore water, or NaSCN.

Whole *D. magna* (usually five per treatment) were removed from the tests and mounted on carbon planchets. A 1:1 (v/v) mixture of Tissue Tek and graphite was used to mount the daphnids on the carbon planchets and to attach the carbon planchets to electron microscopy stubs. The Tissue Tek/graphite mixture did not interfere with subsequent X-ray analysis of *D. magna* (R. Hoke, unpubl. data). For preliminary experiments with post-$NaHCO_3$ exposure organisms, we used traditional cryogenic preparation and fracturing techniques (EM Scope 2000) with etching at −20°C for 15 min if ice crystals were present in the sample (Boekestein et al. 1980; Marshall 1988). Because these fracturing techniques were cumbersome and frequently displaced organisms from the planchets, in subsequent experiments, organisms were mounted on planchets and then plunged into liquid nitrogen for 15 s. The carapace of each *D. magna*

was then gently sliced away with a razor blade to reveal the internal body structure and facilitate penetration of the X-ray beam.

X-ray microanalyses were performed with a JEOL JSM-35C scanning electron microscope equipped with a cryostage and a Tracor Northern Series II X-ray microanalysis system with software. Each measurement was acquired at an accelerating voltage of 20.0 keV for a present scanning time of 60 s. Results are reported as *P/B* ratios (Boekestein et al. 1980; Marshall 1988; Roomans 1988b). Elemental measurements were conducted at five randomly chosen locations within the head and upper body area for each of five *D. magna* from control and either low and high or low, medium, and high experimental treatments. Absolute peak height or emission intensity data were not used due to the lack of analytical standards in an appropriate matrix. The same analytical constraints were operative among all measurements conducted as part of each experiment; therefore, *P/B* ratios and changes in these ratios as a result of experimental treatments, relative to controls, were the data of interest in all experiments.

Data Analysis

X-ray dispersive microanalysis results were analyzed with a nested, fixed effects ANOVA design (Petersen 1985) using the Statistical Analysis System (SAS 1985) software. For the ANOVA, the X-ray microanalysis measurements were nested within experimental units (i.e. individual *D. magna*). In the variance component analysis, three separate aspects of experimental variance were examined: (1) treatment variance, (2) organism (among daphnid within treatment) variance, and (3) measurement (among measurement within daphnid) variance.

Results

Toxicity of Na^+ and HCO_3^-

Forty-eight-hour LC50 values for *D. magna* and *C. dubia* tests with $NaHCO_3$ and NaCl are presented in Table 2. Based on mean LC50 values, *C. dubia* neonates (<24 h old) were approximately 6 times more sensitive to Na^+ as NaCl than *D. magna* neonates of similar age. However, *C. dubia* neonates were only slightly more sensitive (~1.2 times) than *D. magna* neonates to Na^+ as $NaHCO_3$. Little difference was observed in the toxicity of the two compounds to <24-h-old *C. dubia* neonates, but *D. magna* neonates were approximately 5.4 times more sensitive to Na^+ as $NaHCO_3$ than as NaCl. Based on literature values for adults (Dowden 1961), *D. magna* neonates were approximately 1.4 times less sensitive than adults to NaCl whereas adults were approximately 1.4 times less sensitive to $NaHCO_3$ (Table 2).

Calculation of Free CO_2 and HCO_3^-

The results of theoretical equilibrium concentration calculations for CO_2 and HCO_3^- in the AOC sediment pore water samples are presented in Table 1. Calculated concentrations of CO_2 ranged from 0.0 to 1.5 mmol/L, and calculated HCO_3^- concentrations ranged from 1.2 to 29.9 mmol/L.

X-Ray Dispersive Microanalysis

Mean *P/B* ratios (±SD) for Na^+, Si^{4+}, Cl^-, and Ca^{2+} in daphnids analyzed from each experiment are presented in Table 3. Within an experiment and within the individual treatments comprising an experiment, relative levels of Ca^{2+} were the most variable parameter measured based on standard deviation of the means whereas Si^{4+} was the least variable. The variability of relative levels of Na^+ and Cl^- among experiments was similar, but Cl^- levels were generally more variable within an experiment. The results of variance component analyses from the ANOVA's of the X-ray microanalysis data are presented in Table 4. The proportion of the total variance accounted for by among-treatment effects is presented as well as the variance accounted for by among-organism (among-daphnid) effects within an experimental treatment and the among-measurement (measurements within an individual daphnid) variance. Differences in the Cl^- *P/B* ratio among treatments were statistically significant in each experiment. *P/B* ratios for Na^+ and Ca^{2+} also were significantly different among treatments in the No. 3 $NaHCO_3$ experiment, as were the Si^{4+} *P/B* ratios in the Location UG-9 pore water experiment. Variation among organisms treated alike also was significant for Na^+, Si^{4+}, and Cl^- in the No. 3 $NaHCO_3$ and NaSCN experiments and for Cl^- and Ca^{2+} in the Location UG-9 pore water experiment. Treatment effects accounted for 1.1–18.7, 0.0–19.3, 24.8–86.9, and 0.0–21.5% of the total experimental variation in *P/B* ratios for Na^+, Si^{4+}, Cl^-, and Ca^{2+}, respectively. With the exception of experiment No. 4 with the UG-9 pore water, treatments effects accounted for most of the observed variation in Cl^- in each experiment. Among-daphnid variation in *P/B* ratios within a given experimental treatment was always the smallest component of the observed experimental variation in Cl^-. Variance due to differences in measured Cl^- *P/B* ratios within an individual daphnid generally accounted for 12–57% of the total observed experimental variation in *P/B* ratios for Cl^-.

Discussion

Sediment pore water has been hypothesized to be the primary route of exposure to sediment contaminants for benthic macroinvertebrates (Nebeker et al. 1984; Knezovich and Harrison 1988). Pore water also has been demonstrated to be a reasonable surrogate test fraction for the assessment of bulk sediment toxicity (Ankley et al. 1991). Little research has been conducted, however, on the physical or chemical properties of pore water, other than contaminants, which potentially could affect the results of pore water toxicity tests. Numerous characteristics of pore water (major ion content, ratios, etc.) potentially can confound the interpretation of results from pore water toxicity assays.

From our examination of the effects of HCO_3^- concentrations on *D. magna*, it appears that HCO_3^- (or bicarbonate alkalinity) is one factor which can affect toxicity test results, irrespective of the effects of other substances present in the sample. Several indirect lines of evidence lead to this conclusion. The first is that HCO_3^- itself may be partially responsible for the toxicity observed in $NaHCO_3$ reference toxicant assays, as evidenced by the observation that Na^+ as $NaHCO_3$, is approximately 6 times more toxic to <24-h-old *D. magna* than is Na^+ as NaCl. This observation assumes that Cl^- does not protect against Na^+ toxicity. This assumption is supported by recent research which indicates that the toxicity of sodium compounds is due to the anion and not Na^+. Therefore, not only is Cl^- not protective, but it appears that it is responsible for the toxicity of NaCl (D. Mount, ENSR, Inc., Ft. Collins, CO, pers. comm.; Gulley et al. 1991). Recent research by Cowgill and Milazzo (1991) has also reported on alkalinity toxicity to cla-

TABLE 3. Results of X-ray microanalysis experiments with *D. magna*. Values reported are mean (SD) *P/B* ratios for each experimental treatment. Significant treatment effects (indicated with an asterisk) for elemental *P/B* ratios were based on a significant ANOVA for the individual element ($\alpha = 0.05$) followed by Bonferroni *t*-test which indicated a significant difference from either the control or lowest experimental treatment.

Experiment No., toxicant; no. of measurements per daphnid, no. of daphnids per treatment	Treatment	Mean *P/B* ratio			
		Na^+	Si^{4+}	Cl^-	Ca^{2+}
No. 1, $NaHCO_3$; 5, 1	Control	0.30 (0.09)	0.23 (0.04)	0.67 (0.22)	0.82 (0.49)
	11.5 mmol HCO_3^-/L	0.58*(0.15)	0.24 (0.09)	0.25*(0.11)	0.60 (0.48)
	35.9 mmol HCO_3^-/L	0.41 (0.15)	0.25 (0.19)	0.27*(0.06)	0.68 (0.78)
No. 2, $NaHCO_3$; 5, 2	0.4 mmol HCO_3^-/L	0.32 (0.19)	0.25 (0.12)	0.61 (0.13)	2.58 (1.25)
	11.5 mmol HCO_3^-/L	0.47 (0.19)	0.21 (0.09)	0.72 (0.20)	2.49 (1.17)
	35.9 mmol HCO_3^-/L	0.47 (0.29)	0.20 (0.15)	0.00*(0.07)	1.59 (0.98)
No. 3, $NaHCO_3$; 5, 5	Control	0.28 (0.17)	0.13 (0.11)	0.33 (0.18)	0.96 (0.89)
	6.4 mmol HCO_3^-/L	0.18 (0.11)	0.12 (0.06)	0.27 (0.14)	0.96 (0.86)
	11.5 mmol HCO_3^-/L	0.36 (0.19)	0.18 (0.20)	0.76*(0.45)	2.03*(1.32)
No. 4, UG-9 pore water; 5, 5	Control	0.29 (0.20)	0.16 (0.10)	0.62 (0.32)	2.92 (1.88)
	32% pore water	0.26 (0.19)	0.07*(0.06)	0.38*(0.25)	2.60 (2.15)
	100% pore water	0.20 (0.15)	0.17 (0.14)	0.32*(0.11)	2.40 (1.26)
No. 5, NaSCN; 5, 5	Control	0.29 (0.12)	0.11 (0.10)	0.76 (0.22)	0.58 (0.44)
	500 μm NaSCN/L	0.16 (0.13)	0.14 (0.15)	0.32*(0.13)	0.34 (0.33)
	1500 μm NaSCN/L	0.23 (0.11)	0.16 (0.07)	0.40*(0.17)	0.59 (0.52)
	5000 μm NaSCN/L	0.29 (0.11)	0.18 (0.11)	0.44*(0.17)	0.63 (0.44)

TABLE 4. Variance component analyses of results from ANOVA of X-ray microanalysis data.

Experiment No.[a], toxicant	Source	df	% total variance[b]			
			Na^+	Si^{4+}	Cl^-	Ca^{2+}
No. 2, $NaHCO_3$	Total	29	100.0	100.0	100.0	100.0
	Treatment	2	9.8	0.1	86.9*	7.3
	Organism	3	0.0	0.0	0.6	10.1
	Measurement	24	90.2	99.9	12.5	82.6
No. 3, $NaHCO_3$	Total	74	100.0	100.0	100.0	100.0
	Treatment	2	18.7*	0.0	40.5*	21.5*
	Organism	12	19.3*	19.5*	24.6*	11.6
	Measurement	60	62.0	80.5	34.9	66.9
No. 4, UG-9 pore water	Total	74	100.0	100.0	100.0	100.0
	Treatment	2	1.1	19.3*	24.8*	0.0
	Organism	12	5.4	0.0	18.3*	40.2*
	measurement	60	93.5	80.7	56.9	59.8
No. 5, NaSCN	Total	99	100.0	100.0	100.0	100.0
	Treatment	3	13.8	0.0	53.2*	5.2
	Organism	16	17.5*	44.9*	8.3*	0.0
	Measurement	80	68.7	55.1	38.5	94.8

[a]Variance component analysis was not possible on the results of experiment 1 due to inadequate replication.

[b]An asterisk denotes a significant ANOVA *F* value at $\alpha = 0.05$.

docerans. These authors conducted a series of experiments with $NaHCO_3$ as a reference toxicant to determine the chronic toxicity of alkalinity as milligrams $CaCO_3$ per litre to *D. magna* and *C. dubia*. They also reported alkalinity 48-h LC50 values for *D. magna* and *C. dubia* of approximately 800 and 500 mg/L, respectively. These values compare well with the mean 48-h *D. magna* and *C. dubia* LC50 values of 921 mg/L (15.1 mmol/L) and 781 mg/L (12.8 mmol/L), respectively (Table 2), reported in this study.

A second line of evidence is based on theoretical equilibrium calculations of HCO_3^- concentrations in the AOC pore waters. Based on these calculations and the cladoceran LC50 values for HCO_3^- determined in this study, concentrations of HCO_3^- sufficient to produce observable toxicity were present in several of the AOC sediment pore water samples (Locations UG-8, 9, 11, and 12). Based on the lack of effects on *C. dubia* at CO_2 concentrations of 0.014 mol/L (600 mg/L) in acute tests (Mount and Anderson-Carnahan 1988), the calculated CO_2 concentrations in the AOC sediment pore water samples were not great enough to cause observable effects. The final evidence for the toxic effects of HCO_3^-, and its proposed mode of action, comes from the results of the X-ray microanalysis.

The choice of X-ray dispersive microanalysis of elemental concentrations in *D. magna* tissues was based on the hypoth-

esized mode of action for HCO_3^-. It is well known that freshwater fish actively regulate internal concentrations of several ions by exchanging them for other ions contained in the external medium (Garcia-Romeu and Maetz 1964; Maetz and Garcia-Romeu 1964; Garcia-Romeu et al. 1969; de Renzis and Maetz 1973; de Renzis and Bornancin 1977; Payan 1978). The existence of a similar active regulatory mechanism in *D. magna* for metabolically produced H^+ and external Na^+ was proposed by Potts and Fryer (1979). They hypothesized that freshwater crustaceans obtain a large portion of their Na^+ by active uptake from water. Higher rates of Na^+ turnover (0.163/h) were found for *D. magna* by Potts and Fryer (1979) compared with rates for freshwater fish or crayfish weighing 10–100 g (0.001–0.005/h), but rates were believed to be consistent with the greater cladoceran surface/volume ratios. Similar Na^+ turnover rates for *D. magna* were observed by Stobbart et al. (1977) in experiments in which they demonstrated that (1) Na^+ influx in *D. magna* followed a Michaelis–Menten relationship, (2) the Na^+ transport mechanism can be saturated at Na^+ concentrations $\geq$0.5 mmol/L, and (3) the metabolic inhibitors KCN and dinitrophenol (DNP) inhibited Na^+ uptake at less than acutely lethal concentrations. They also suggested that the presence of a Michaelis–Menten type uptake relationship for Na^+ and the effects of KCN and DNP on Na^+ influx indicated that Na^+ uptake is actively regulated in *D. magna*. Although the presence of actively regulated HCO_3^-–Cl^- exchange similar to that found in freshwater fish has never been demonstrated in *D. magna*, it has been suggested that the NH_4^+–Na^+ and HCO_3^-–Cl^- exchange processes may occur frequently in freshwater animals (Maetz and Garcia-Romeu 1964).

The results of our experiments with $NaHCO_3$, AOC Location UG-9 pore water, and the metabolic inhibitor NaSCN can be interpreted as supportive of the hypothesis that the exchange process for HCO_3^-–Cl^- is actively regulated in *D. magna* and that the mode of action for HCO_3^- toxicity is the inhibition of this exchange process which results in decreased internal concentrations of Cl^-. This study and others (Cowgill and Milazzo 1991; Gulley et al. 1991) have demonstrated that HCO_3^- can be toxic to *D. magna* and *C. dubia*. This study also has demonstrated that *D. magna* from HCO_3^- treatments which caused acute toxicity exhibited decreased internal Cl^- concentrations, relative to controls, based on mean *P/B* ratios from X-ray microanalysis. Calculations of the theoretical equilibrium concentration of HCO_3^- in AOC Location UG-9 pore water demonstrated that the sample contained sufficient HCO_3^- to cause acute toxicity based on the 48-h LC50 values determined in the $NaHCO_3$ reference toxicant assays. X-ray microanalysis demonstrated that organisms from the AOC Location UG-9 pore water toxicity test exhibited decreased internal Cl^- concentrations, relative to control, in the 32 and 100% pore water exposures. The metabolic inhibitor NaSCN also caused statistically significant ($\alpha = 0.05$) reductions in internal concentrations of Cl^- across all NaSCN treatments, indicating that the uptake of Cl^- from the external environment is actively regulated in *D. magna*.

The nested ANOVA experimental design facilitated the evaluation of the variance in measured elemental *P/B* ratios due to (1) experimental treatment, (2) among-daphnid differences within a treatment, and (3) among-measurement differences within an individual daphnid (Table 4). With the exception of experiment No. 4 with AOC Location UG-9 porewater, treatment effects accounted for the majority of the variation in measured *P/B* ratios for Cl^- in *D. magna*. In experiment No. 4. with the Location UG-9 porewater, treatment effects accounted for 25% of the observed variation in Cl^- *P/B* ratios. Among-measurement differences within the same daphnid accounted for the next greatest proportion of the experimental variance in *P/B* ratios for Cl^- whereas among-daphnid differences always accounted for the smallest proportion of the experimental variance in *P/B* ratios for Cl^-. This analysis highlights the importance of the experimental treatments on *P/B* ratios for Cl^- in *D. magna*. It further suggests that in future investigations of this type, greater experimental precision could be achieved by decreasing the number of daphnids analyzed per experimental treatment and increasing the number of X-ray microanalysis measurements conducted on an individual daphnid.

Additional circumstantial support for the hypothesis that HCO_3^-–Cl^- exchange is actively regulated in other aquatic organisms may be found in the field studies of Montezuma Well, Arizona, conducted by Blinn and Sanderson (1989) and Dehdashti and Blinn (1991). In this ecosystem, high concentrations of CO_2 (>550 mg/L) and alkalinity (>600 mg/L as $CaCO_3$) were hypothesized to be responsible for the lack of fish and benthic macroinvertebrates of the orders Tricoptera, Lepidoptera, Megaloptera, and Anisoptera. Limited collections of benthic macroinvertebrates in the orders Chironomidae and Ephemeroptera were attributed to the same phenomenon. Planktonic rotifers and cladocerans also were absent from this ecosystem. Field studies by Winget and Mangum (1979) concluded that the number of macroinvertebrate taxa in 164 samples from 20 different streams was inversely correlated ($r = -0.67$, $p = 0.001$) with ambient stream alkalinity as $CaCO_3$. Similarly, McCarraher and Thomas (1968) observed that in alkaline lakes of Nebraska, fathead minnows (*Pimephales promelas*) fluorished when carbonate alkalinity was below 800 ppm and total alkalinity was below 1800 ppm. However, carbonate or total alkalinity in excess of these values was reported to greatly impair reproduction and abundance of this species.

The proposed existence in *D. magna* of an actively regulated exchange process for HCO_3^- and Cl^- and the hypothesis that disruption of this process is the mode of action for HCO_3^- toxicity have several practical implications. HCO_3^- concentrations should be routinely measured during toxicity tests of aqueous samples, such as pore waters or effluents. If HCO_3^- concentrations are sufficient to produce toxicity, this observation should be considered when interpreting the test results. The importance of HCO_3^- toxicity also needs to be addressed in toxicity identification evaluation (TIE) procedures (Mount and Anderson-Carnahan 1988, 1989; Mount 1989), both for effluents and sediment pore waters, because current TIE methodology would not identify high HCO_3^- concentrations as the specific cause of observed toxicity. Instead, the observed toxicity could be wrongly ascribed to the effects of total dissolved solids (TDS). This would implicate cations or cation ratios in the sample as the cause of observed effects when, in fact, the effects were due to the anion HCO_3^-. Finally, since active regulatory processes for HCO_3^-–Cl^- also exist in freshwater fish and amphibians, and may exist for other aquatic invertebrates, TIE procedures performed with these species also may be affected by high HCO_3^- concentrations in the test solution.

Acknowledgements

The authors would like to thank Jane Thompson, Jane Norlander, and Debra Williams for typing various drafts of the manuscript. Mary Schubauer-Berigan, Teresa Norberg-King, Gary Ankley, Howard McCormick, Don Mount, and an anonymous reviewer provided helpful comments on earlier versions of the manuscript.

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Linkage between Epilithic Algal Growth and Water Column Nutrients in Softwater Lakes

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We examined the dependence of epilithic algal standing crop, production, and nutrient limitation upon water column nutrients in 12 softwater lakes of northeastern Pennsylvania. Elevated dissolved inorganic nitrogen accompanied low dissolved inorganic carbon in the more acidic lakes, while P varied little within the study area. The growth of epilithon on clay flower pot substrata diffusing combinations of N ($NaNO_3$), P (Na_2HPO_4), and C ($NaHCO_3$) was compared with growth on control substrata to evaluate which of the three nutrients limited growth in each lake. Standing crop accrual as chlorophyll *a* on control substrata averaged 0.8 μg/cm², with little variation among lakes. Nutrient limitation of growth, however, was strongly related to lake alkalinity. Chlorophyll *a* was typically enhanced by N and/or P only in lakes with alkalinity greater than ~100 μeq/L and responded strongly to C enrichment in the two most acidic lakes. Combined addition of all three nutrients produced the largest chlorophyll *a* accrual in all 12 lakes. Invertebrate grazer biomass, dominated by chironomids in the more acidic lakes and by snails at higher alkalinity, was negatively related to chlorophyll *a* on these NPC substrata ($r = -0.57$, $p = 0.05$) and may have reduced algal standing crop well below nutrient-sustainable levels in some lakes.

Nous avons examiné la dépendance de la biomasse des algues épilithiques, leur production et la limitation de leur croissance par les éléments nutritifs de la tranche d'eau dans 12 lacs d'eau douce du nord-est de la Pennsylvanie. Des concentrations élevées d'azote inorganique dissous accompagnaient de faibles concentrations de carbone inorganique dissous dans les lacs plus acides, tandis que la teneur en P variait peu à l'intérieur de la région étudiée. On a comparé la croissance de l'épilithon sur des substrats de pots de fleurs en argile diffusant une combinaison de N ($NaNO_3$), P (Na_2HPO_4) et C ($NaHCO_3$) à la croissance sur des substrats témoins afin d'évaluer quel élément nutritif parmi les trois limitait la croissance dans les lacs. L'accumulation de la biomasse sous la forme de chlorophylle *a* sur les substrats témoins était en moyenne de 0,8 μg/cm², avec peu de variations d'un lac à l'autre. Cependant, la limitation de la croissance par les éléments nutritifs était fortement reliée à l'alcalinité du lac. La teneur en chlorophylle *a* n'était typiquement augmentée par N ou par P que dans les lacs où l'alcalinité était supérieure à environ 100 μéq/L et réagissait fortement à l'enrichissement en C dans les deux lacs les plus acides. L'addition combinée des trois éléments nutritifs a produit l'accumulation de chlorophylle *a* la plus importante dans l'ensemble des 12 lacs. On a noté une relation négative entre la biomasse des invertébrés herbivores, dominés par les chironomidés dans les lacs les plus acides et par les gastéropodes dans les lacs plus alcalins, et la chlorophylle *a* sur ces substrats de NPC ($r = -0,57$, $p = 0,05$), ce qui peut avoir réduit la biomasse des algues bien en dessous des concentrations durables sur le plan des éléments nutritifs dans certains lacs.

Received August 28, 1991
Accepted February 10, 1992
(JB205)

Reçu le 8 août 1991
Accepté le 10 février 1992

Observations of the frequent proliferation of benthic algae near sources of nutrient input to lakes (e.g. Ennis 1975) and in response to whole-lake nutrient enrichment (e.g. Shortreed et al. 1984) have long supported the view that the growth of attached algae in the littoral zone is often limited by nutrient supply. A number of alternative nutrient sources are recognized. Epipelic (Stevenson et al. 1985; Hansson 1989) and epiphytic (Moeller et al. 1988) communities, for example, may in some instances receive substantial nutrition from the substratum. Epilithic assemblages on chemically inert surfaces are, by contrast, usually thought to obtain their nutrients primarily via diffusion from the water column. Cattaneo's (1987) demonstration that standing crop of shallow-water epilithon at 13 lake sites in Quebec was positively correlated with total P (TP) in the water column is consistent with this view.

At the same time, Cattaneo's (1987) work clearly showed that the linkage between water column nutrients and algal standing crop was much weaker for the epilithon than for the phytoplankton. Recent studies of the fine structure and chemical environment of the benthic algal mat (Lock et al. 1984; Riber and Wetzel 1987; Sand-Jensen 1989; Bothwell 1988, 1989) likewise suggest that nutrients actually available to algal cells within the mat may be dramatically reduced compared with concentrations available to the phytoplankton. Nutrients regenerated within the mat by grazing or decomposition may thus increasingly determine growth as standing crop itself increases (Mazumder et al. 1989; Paul et al. 1989; Mulholland et al.

1991). If different nutrients are retained or internally recycled at different rates, then the degree of nutrient linkage with the water column must be viewed as weak and indirect.

Softwater lakes displaying a range of alkalinity provide excellent settings for examining nutrient control of epilithic algal growth, as inorganic C, N, and P may all be found in potentially limiting supply. In more acidic lakes, for example, inorganic N may accumulate in the water column owing to high levels of atmospheric deposition and reduced efficiency of N utilization by bacteria (Kelly et al. 1990) in the presence of a P-limited phytoplankton (Rudd et al. 1990). Sulfate loading may also increase sedimentary release of soluble reactive P (SRP) (Caraco et al. 1989). Dissolved inorganic C (DIC) concentrations may at the same time decline to levels known to limit benthic algal photosynthesis (Turner et al. 1987). These processes can greatly modify ratios of C:N:P in the water column.

The Redfield (1958) ratio of 106C:16N:1P (6.6C:1N), approximating the relative nutrient content of algae growing under nutrient-sufficient conditions, may provide a crude but reasonable first step in predicting a limiting nutrient based upon ambient water chemistry (see Suttle and Harrison (1988) and Elrifi and Turpin (1992) for recent discussions of factors causing variation from the Redfield ratio). If ratios of C:N:P in the water column accurately predict the nutrient which actually limits growth of the epilithon, then strong and direct linkage is implied.

The assemblage of invertebrate grazers associated with the epilithon is also known to be affected by lake alkalinity. In particular, snails usually disappear under acid conditions (Økland 1980) whereas chironomids may become increasingly abundant (Wiederholm and Eriksson 1977; Raddum and Saether 1981). One effect of algal consumption by grazers may be to "decouple" algal production from standing crop, thereby weakening the perceived relationship of standing crop to water column nutrients. Other factors such as erosional scour may also strongly influence standing crop (Cattaneo 1990) but are not directly related to the alkalinity gradient considered here.

In this study, we extend Cattaneo's (1987) lake comparison approach by examining epilithic algal growth in 12 softwater lakes displaying a range in alkalinity and markedly different ratios of ambient C:N:P.

(1) We relate standing crop accrual on artificial substrata to nutrients in the water column and also measure variation in photosynthesis on the substrata in relation to changes in DIC and light.

(2) We identify the limiting nutrient(s) in each lake using enrichment bioassays (substrata diffusing combinations of C, N, and P) and relate these results to predictions of nutrient limitation based upon C:N:P in the water column.

(3) We examine the degree to which invertebrate grazing may reduce epilithic algal standing crop below nutrient-sustainable levels.

Materials and Methods

Study Area

The lakes chosen for study are all situated in northeastern Pennsylvania. They collectively represent maximum variation in pH and alkalinity of the natural lakes within the region, based upon an earlier comprehensive survey of water chemistry (J. W. Sherman, unpubl. data). The lakes vary in size from 13.4 to 55.9 ha in surface area and from 3.6 to 22.2 m in depth. The study was restricted to clearwater lakes with low dissolved color (<25 Pt-Co units) to reduce the potential influences of complex organic compounds (e.g. humates) and light limitation on epilithic algal growth. Eleven of the 12 lakes are located in adjacent Wayne and Pike counties. Sly (SL), Rock (RO), and Swago Pond (SW) in northern Wayne County are more alkaline, while lakes in central Pike County (Earnest (EA), Giles (GI), Laura (LA), Little Brink (LB), Little Logtavern (LL), and Silver (SI)) are among the most acidic in the two-county area. First Pond (FI) and Waynewood (WA) have higher alkalinity and nutrient concentrations than might be expected from their location and morphometry, apparently owing to prolonged cultural eutrophication (J. W. Sherman, unpubl. data). Ganoga Lake (GA), in Sullivan County approximately 125 km to the west, was added to increase the representation of lakes with very low pH and alkalinity.

Nutrient-Diffusing Substrata

Clay flower pot substrata were soaked for 3 d in 10% HCl and an additional 3 d in deionized water to reduce nutrient contamination and then filled with 2% agar and combinations of 0.5 mol $NaNO_3$ (N)/L, 0.05 mol Na_2HPO_4 (P)/L, and 60 g $NaHCO_3$ powder (C)/pot. Methods of substratum construction and nutrient release rates are described in previous work (Fairchild et al. 1989). Four replicates of each of eight nutrient treatments (CONT (= agar only), N, P, C, NP, PC, NC, NPC) were placed in each lake. The substrata were positioned approximately 0.5 m apart in four rows, each with all eight treatments. The same randomized block design was used in all 12 lakes. All sites were in shallow water (0.3–0.7 m) with little or no aquatic vascular plant cover. The surrounding sediments ranged from flocculent silt or detritus to firm sand or rocks.

The duration of substratum incubation was 36–40 d in all but two of the lakes. Substrata were examined after 45–46 d in EA and after 32–33 d in FI. Prior to retrieval, rates of photosynthesis were determined for the CONT, NP, C, and NPC substrata in five of the lakes. Plexiglas® metabolic chambers (volume = 0.2 L) were attached to the substrata (Fairchild and Everett 1988), and the enclosed epilithon was incubated in the dark for an average of 2.1 h (SD = 0.4 h) to obtain community respiration (CR) by microwinkler determinations of dissolved oxygen decline. A subsequent light incubation ($\bar{x}$ = 1.0 h, SD = 0.4 h) provided estimates of net productivity (P_n). Gross productivity was calculated as the sum of P_n and CR. Light was measured at 10-min intervals with a LI-COR 192SA quantum sensor placed directly above the sediments. Light reaching the substrata was presumed less than but proportional to light outside the chambers. Photosynthetic measurements were taken concurrently for the four substrata (treatments) in a given row of the randomized block design to ensure similarity of light and temperature effects. Completion of all measurements required 2–3 d per lake, however, and usually involved a range in temperature and light levels.

On retrieval of each substratum, all associated material was removed using a knife and hard-bristled toothbrush and partitioned for analysis of chlorophyll *a*, algal species composition, and invertebrate grazer biomass. Chlorophyll *a* was extracted in hot ethanol and measured with a Bausch and Lomb 501 spectrophotometer. Samples were acidified for separate phaeophytin determination (Nusch 1980).

Ten-millilitre aliquots of each sample were preserved with 1 or 1.5 mL of glutaraldehyde. Algae were enumerated at 430× until at least 500 counting units (filament segments, unicells, colonies) were identified. Cell volumes were estimated for each

taxon and multiplied by cell densities to compute taxon biovolumes (Fairchild and Everett 1988).

Dry weight biomass of invertebrates associated with the periphyton was estimated for only four treatments (CONT, C, NP, and NPC substrata). Each sample was first passed through a 64-μm-mesh sieve and then placed in a Bogurov counting chamber (Gannon 1971). Invertebrates were identified, dried at 65°C, and then weighed using a Cahn 31 microbalance. All larger invertebrates (e.g. Isopoda, Gastropoda, Trichoptera, Ephemeroptera) were weighed, either individually or together by taxonomic group. Snail weights were multiplied by 0.2 to estimate tissue dry mass (Cattaneo and Kalff 1986). Biomass of smaller invertebrates which were consistently abundant in the samples (e.g. chydorid Cladocera, Chironomidae) was estimated by weighing a random subset of individuals counted (usually 20–50), computing mean weight per individual, and then multiplying mean weight by the total count. Mean weights per individual of small-bodied taxa which were rarely encountered (e.g. Harpacticoida) were either estimated from direct measurements or obtained from literature values (e.g. Downing and Rigler 1984). Soft-bodied microinvertebrates such as rotifers, nematodes, and protozoans were occasionally seen but ignored, as most were presumed to have been lost during sieving. Primarily carnivorous taxa (e.g. Odonata, Acari) and zooplankton (e.g. calanoid Copepoda) were also excluded from the counts.

Water Chemistry

Water samples were collected twice from each lake, at the beginning and end of the substratum incubation period. Samples were either filtered and frozen (PO_4, NO_3, NO_2, NH_3), frozen without filtering (total Kjeldahl N, TP), or filtered and refrigerated (soluble reactive SiO_2) until analysis. All analyses were performed using a Technicon II autoanalyzer.

Temperature and pH were measured in the field on both dates using an Orion model 250 pH meter. Alkalinity was determined by Gran titration. Samples for measurement of total DIC were obtained once for each lake, at or close to the time when the substrata were collected, and analyzed by gas chromatography within 1 d using the method of Stainton (1973).

Statistical Analysis

The data were analyzed using SAS (SAS Institute, Inc. 1982). Trends in mean nutrient concentrations and in mean chlorophyll *a* levels on control substrata were related both to each other and to mean lake alkalinity using Pearson product-moment correlations (r). Because the substratum incubation period varied among lakes, we also used a Pearson correlation to check for potential effects of colonization time on chlorophyll *a* accrual. We evaluated nutrient enrichment effects on chlorophyll *a* in each lake using one-way ANOVA and the Student–Newman–Keuls (SNK) comparison of treatment means.

Photosynthetic rates (P_g) were initially compared by nutrient treatment in each lake using one-way ANOVA and the SNK test. We also used multiple regression to evaluate the influence of three variables, light, DIC, and $\log_{10}$ (chlorophyll *a*), upon P_g of periphyton on each nutrient treatment, with the data from all lakes combined in the analysis.

The proportion of variation in chlorophyll *a* on the NPC substrata associated with variation in grazer biomass (r^2) was determined from a Pearson correlation. Mean chironomid dry weight in the four most acidic lakes without snails was compared with dry weight in the eight lakes with snails using a two-sample t-test.

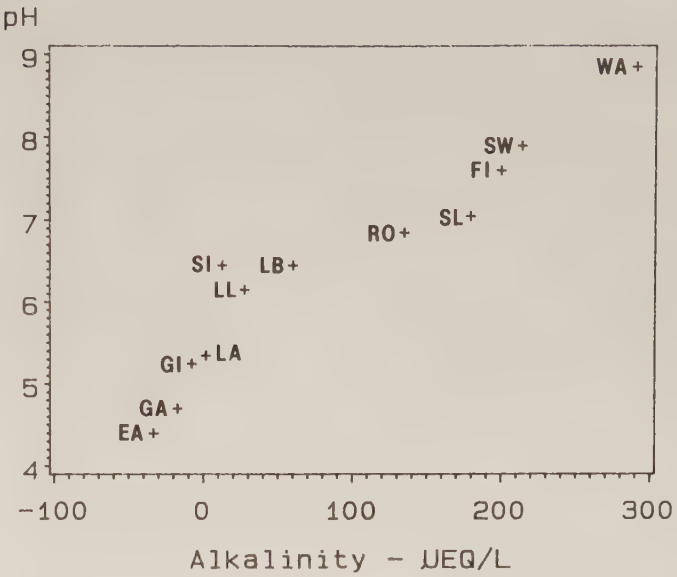

FIG. 1. Mean pH and alkalinity (n = 2) of the 12 lakes, based upon samples collected at the beginning and end of substratum incubation. The average range of within-lake variation in pH was 0.3 unit. The average SE for alkalinity was 6.6 μeq/L.

Results

Water Chemistry

Alkalinity varied widely from −33 to 290 μeq/L (pH = 4.4–8.8) in the 12 lakes (Fig. 1) and was used as the primary variable to which nutrient concentrations and biotic measurements were related. Measurements of DIC varied closely with alkalinity (r = 0.98, $p < 0.001$) and ranged from 10 to 240 μmol/L (Fig. 2a).

Total N (TN = NH_3 + NO_3 + NO_2 + ON) varied from 2.21 μmol/L in LA to 45.1 μmol/L in WA (Fig. 2b). Dissolved inorganic N (DIN = NH_3 + NO_3 + NO_2) reached much higher levels in the two most acidic lakes (10.7 μmol/L in EA, 22.6 μmol/L in GA) than in lakes with higher alkalinity. A pronounced decline in DIN/TN with increasing alkalinity was also apparent (r = −0.72, $p < 0.01$).

Total P (TP = PO_4 + OP) was highest in WA (1.26 μmol/L) and generally increased with increasing TN (r = 0.58, $p < 0.05$) and increasing alkalinity (r = 0.75, $p < 0.01$) (Fig. 2c). Concentrations of PO_4 ranged from 0.06 to 0.22 μmol/L. The proportion PO_4/TP was not significantly related to alkalinity (r = −0.51).

Midsummer SiO_2 concentrations were low throughout the study area (Fig. 2d). Highest concentrations were recorded in WA (31.8 μmol/L) and LL (25.1 μmol/L), with all other lakes exhibiting much lower values.

Periphyton Standing Crop

The algal community on control substrata was dominated in all lakes by Chlorophyta, which comprised 66.1–99.9% of total biovolume ($\bar{x}$ = 89.7%). *Mougeotia* sp. was especially abundant in the six most acidic lakes, while *Oedogonium* sp. and *Stigeoclonium lubricum* were dominant at higher alkalinity. Blue-greens reached maximum relative abundance in two of the more alkaline lakes, FI (6.9%) and WA (5.7%), while diatoms were most abundant in RO (30.2%), LB (13.7%), and SI (14.2%). The rather complex changes in community structure which accompanied changing pH/alkalinity and the different

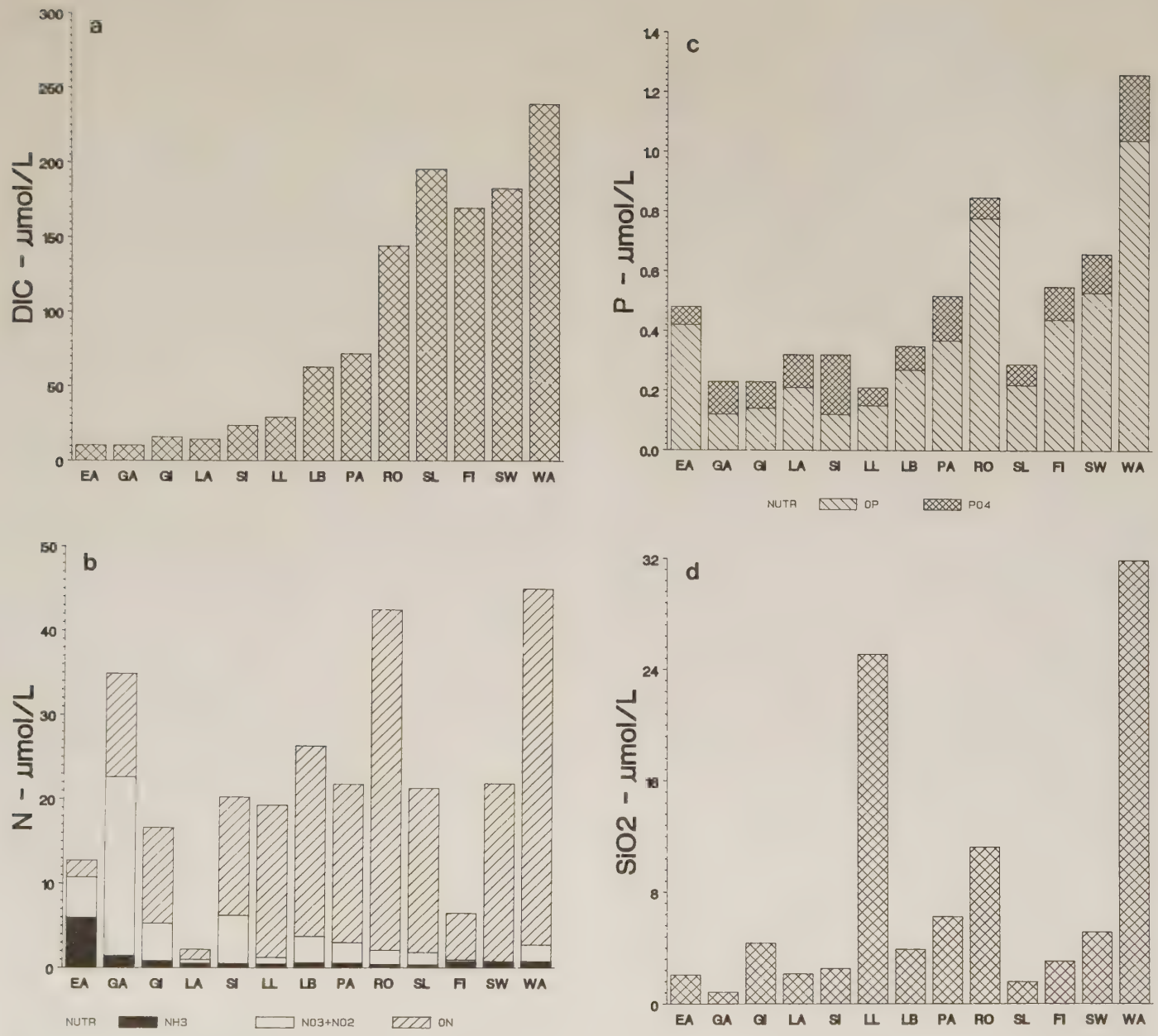

FIG. 2. Concentrations of (a) DIC, (b) N, (c) P, and (d) SiO_2 in the 12 lakes, arranged in order of increasing alkalinity. "OP" and "ON" indicate the organically bound components of those nutrients. Coefficients of variation averaged 20.1% of lake means ($n = 2$) for NH_3, 55.0% for $NO_3 + NO_2$, 25.5% for TN, 41.8% for PO_4, 22.8% for TP, and 33.1% for SiO_2. Values for DIC are single mesurements taken at or very near the time of substratum retrieval.

nutrient enrichments are detailed in a separate paper (G. W. Fairchild and J. W. Sherman, in prep.).

Periphyton chlorophyll *a* responses to the eight nutrient treatments for all lakes are shown in Fig. 3. Mean chlorophyll *a* on control substrata in the 12 lakes ranged from 0.3 to 1.6 ($\bar{x} = 0.8$) µg/cm^2 and was not significantly related to DIC, DIN, or PO_4 (all $p > 0.05$). A slight, negative correlation ($r = -0.59$, $p < 0.05$) was observed between incubation time and chlorophyll *a* accrual, apparently attributable in large part to unusually low chlorophyll *a* levels ($\bar{x} = 0.3$ µg/cm^2) and lengthy substratum incubation in EA.

The failure of standing crop to increase with any of the nutrients across lakes may be explained partly by changes in the limiting nutrient along the alkalinity gradient. Bicarbonate enrichment significantly elevated standing crop above controls (all $p < 0.05$) in the acidic lakes EA, GA, and SI with DIC/DIN <4 (Table 1). In contrast, N or NP addition enhanced growth only in the most alkaline lakes with DIC/DIN ≥69. Growth in lakes with intermediate alkalinity was generally stimulated only when N and C were supplied jointly. The shift from C limitation at DIC/DIN less than the Redfield ratio of 6.6 to N limitation at higher DIC/DIN thus indicates probable uptake in approximate proportion to water column concentrations of those two nutrients. In contrast, despite mean lake TN:TP ratios that typically exceeded the Redfield ratio of N:P = 16 (suggesting probable P limitation), P was not the primary limiting nutrient to standing crop in any of the lakes. We did find either joint NP limitation or secondary limitation by P (defined as significant additional growth compared with that elicited by a primary limiting nutrient) in all 12 lakes, however.

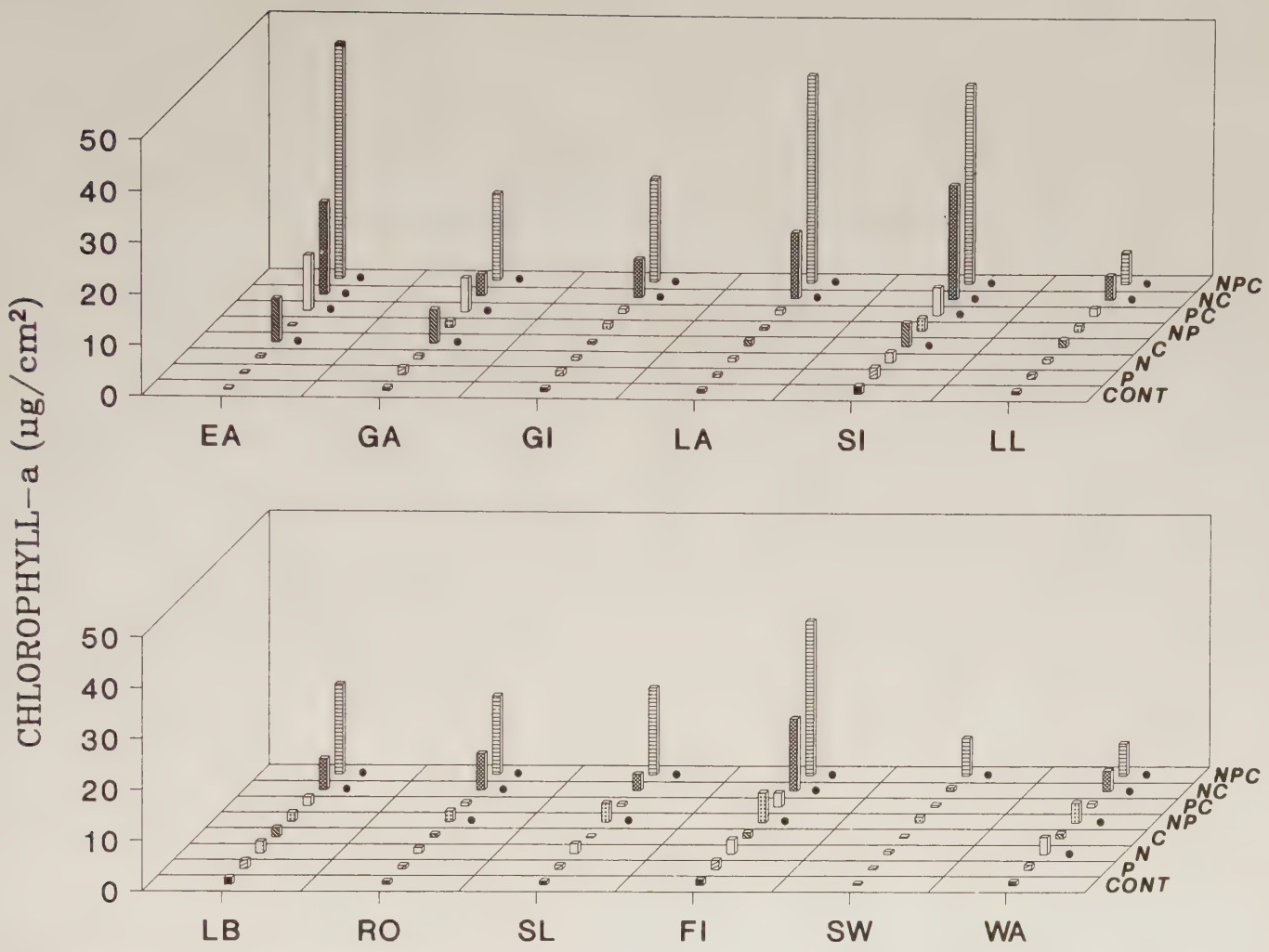

FIG. 3. Mean chlorophyll *a* levels by lake (arranged in order of increasing alkalinity) and nutrient treatment. Nutrient additions that significantly enhanced chlorophyll *a* ($p < 0.05$) relative to control substrata are shown with closed circles to the right of the bar.

TABLE 1. Nutrient limitation of chlorophyll *a* standing crop in relation to ratios of water column nutrients in the 12 lakes, arranged in order of ascending alkalinity. Primary limiting nutrient(s) (PLN) are defined as that nutrient, or (if none) nutrient pair, which significantly enhanced chlorophyll *a* levels above controls ($p < 0.05$). In RO, both NC and NP, but not N, significantly stimulated chlorophyll *a* production.

	EA	GA	GI	LA	SI	LL	LB	RO	SL	FI	SW	WA
DIC/DIN	1.0	0.5	3.0	16	3.8	22	17	69	109	189	261	86
TN/TP	26	154	73	6.9	63	100	74	51	74	12	34	36
PLN	C	C	NC	NC	C	NC	NC	NP/NC	NP	NP	NPC	N

Primary Productivity

Highest rates of photosynthesis accompanied the much higher chlorophyll *a* levels on the NPC substrata in all five lakes examined (Fig. 4). Measurements were often variable within treatments, in part owing to changing light levels, and even large differences among treatment means were sometimes not significant. Results of the multiple regression analysis (Table 2) indicated that P_g on control substrata was largely determined by available DIC. In contrast, photosynthesis on NPC substrata was strongly limited by light rather than DIC owing to the diffusion of bicarbonate from the substrata into the chambers (Fairchild and Everett 1988). P_g also increased significantly with increasing chlorophyll *a* on NP and NPC substrata.

Invertebrate Grazers

Dry weight estimates of the dominant invertebrate taxa are shown in Fig. 5. Snails (chiefly *Amnicola limosa*, *Helisoma*, and *Physa*) were restricted to lakes with >10 µeq/L alkalinity. Most individuals were very small and probably originated as eggs deposited on the substrata. Mean snail (tissue) dry weights nonetheless comprised 30–94% of total measured grazer biomass in the eight lakes where snails were present.

Chironomids, consisting largely of early instar larvae, numerically dominated the insect fauna. Chironomid biomass was approximately four times greater in the four more acidic lakes without snails than in the eight lakes with snails ($p < 0.001$). Other insect taxa, including the herbivorous dipteran

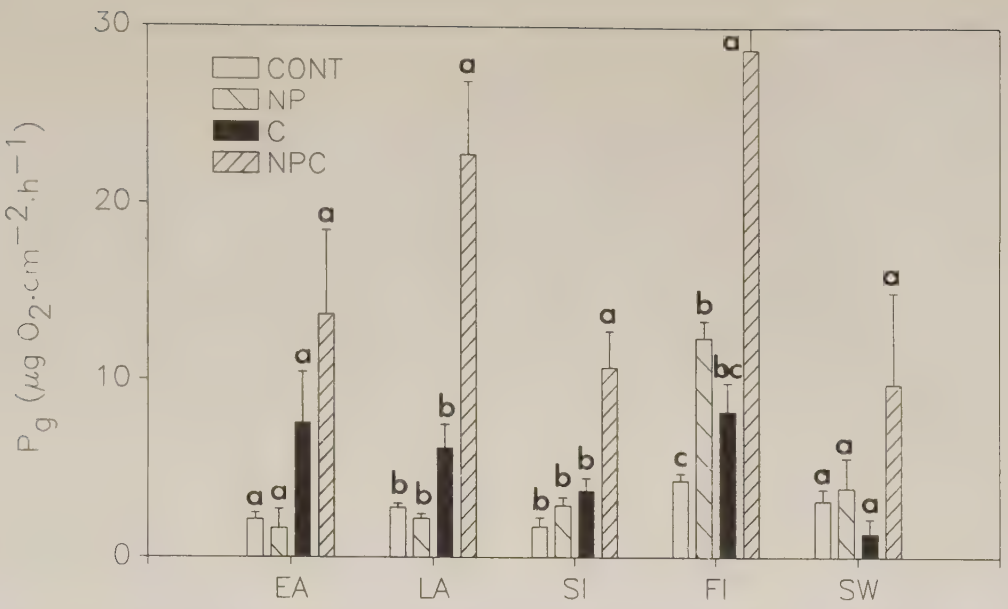

FIG. 4. Primary production (P_g) on substrata diffusing four nutrient combinations in five lakes ($\bar{x} \pm$ SE). Treatment means sharing the same letter within a given lake did not differ significantly ($p > 0.05$).

TABLE 2. Multiple regression analysis of P_g ($\mu g\ O_2 \cdot cm^{-2} \cdot h^{-1}$) response to light ($\mu ein \cdot m^{-1} \cdot s^{-1}$), DIC ($\mu mol/L$), and $\log_{10}$chlorophyll *a* ($\mu g/cm^2$) for four nutrient treatments. Shown are the intercept and slopes, significance levels ($*p < 0.05$, $**p < 0.01$, NS = $p > 0.05$), and number of observations (n). Measurements were combined for five lakes (EA, LA, SI, FI, SW).

Treatment	n	Intercept	Light	DIC	$\log_{10}$(Chl *a*)	R^2
CONT	14	+1.35*	+0.001 NS	+0.009*	+0.178 NS	0.62
NP	16	+1.04 NS	+0.002 NS	+0.026*	+4.207*	0.79
C	16	+0.59 NS	+0.004 NS	+0.006 NS	+3.511 NS	0.28
NPC	16	−23.2 NS	+0.014**	+0.046 NS	+17.33 *	0.72

Culicoides, mayfly, and caddisfly larvae, were generally less abundant than the chironomids. Microcrustacea (Cladocera, Copepoda, Ostracoda) were often numerically abundant, but typically contributed little to total grazer biomass. Isopoda contributed 62.2% of total biomass in EA but were rarely found elsewhere. Oligochaetes and oribatid mites were found consistently at low densities. In effect, a rather pronounced shift in dominance from chironomids to snails and a slight increase in total grazer biomass accompanied higher alkalinity.

Given standardized enrichment with all three nutrients, the same growth potential might be expected in all lakes, with residual variation in actual standing crop attributable to other factors (e.g. sloughing, differences in colonization time, grazing). We found that 33% of the variation in chlorophyll *a* among lakes on the NPC substrata was related to mean grazer biomass ($r = -0.57$, $p = 0.05$) (Fig. 6). The relationship further appeared to be tied to alkalinity. The three most acidic lakes (EA, GA, GI) had the lowest invertebrate biomasses and relatively high chlorophyll *a* densities whereas the most alkaline lakes (WA, SW, SL) all had high invertebrate biomasses and relatively lower chlorophyll *a* densities. A significant relationship between invertebrate biomass and chlorophyll *a* was not observed for the other nutrient treatments. The ratio of algal biomass to grazer biomass on the NPC substrata ranged from 0.3 to 40 ($\bar{x} = 7.0$) μg Chl *a*/μg dry wt (with highest values in the acidic lakes) and was also much greater than for other treatments (combined $\bar{x} = 0.2$ μg Chl *a*/μg dry wt).

Discussion

The epilithon was strongly nutrient limited in all 12 lakes, with substantial growth stimulation accompanying nutrient enrichment. Paradoxically, standing crop on control substrata showed little correlation with water column nutrients, much as in Cattaneo's (1987) previous work. We offer two explanations of the paradox. First, because of changing C:N:P ratios along the alkalinity gradient, no single nutrient regulated growth in all 12 lakes, and a positive correlation with any single nutrient would therefore not be expected. Second, Bothwell (1988, 1989) has shown that water column nutrients may be reduced by as much as two orders of magnitude within the benthic algal mat. What appear to be substantial differences in water column nutrients may thus be reduced to very minor differences in nutrients actually available to cells within the epilithon.

As the volume and aggregate nutrient demand of the epilithon increase, diffusion across the boundary layer cannot increase commensurately, and nutrient supply rates from the water column per unit standing crop must decline (Riber and Wetzel 1987; Stevenson et al. 1991). This decline in access to externally derived nutrients may indeed be so strong as to slow chlorophyll *a* accrual in quiet water to rates easily matched by removal through grazing and sloughing, thereby yielding the consistently low standing crops we observed on the control substrata. Substantial standing crop response to our nutrient treatments was apparently only possible because diffusion from the

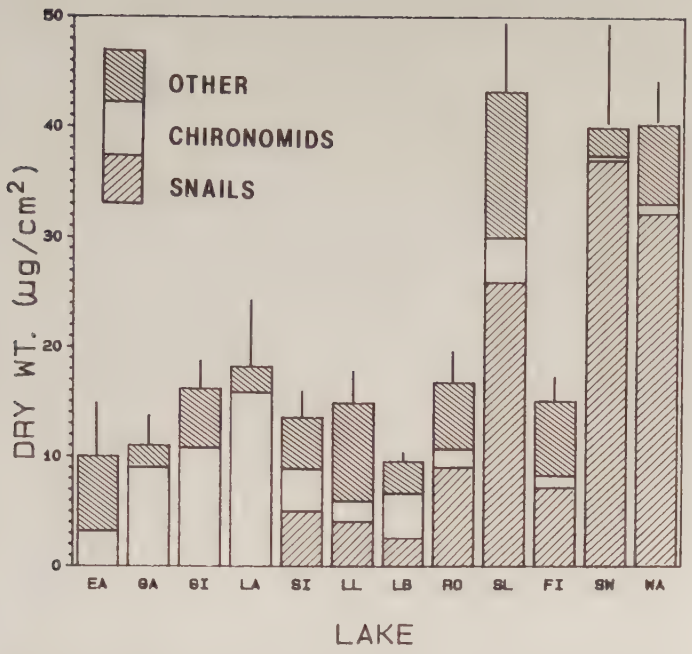

FIG. 5. Mean dry weight (all four treatments combined) of snails, chironomids, and other grazing invertebrates in the 12 lakes, arranged in order of ascending alkalinity. Error bars denote ± SE of the mean estimate of total grazer biomass.

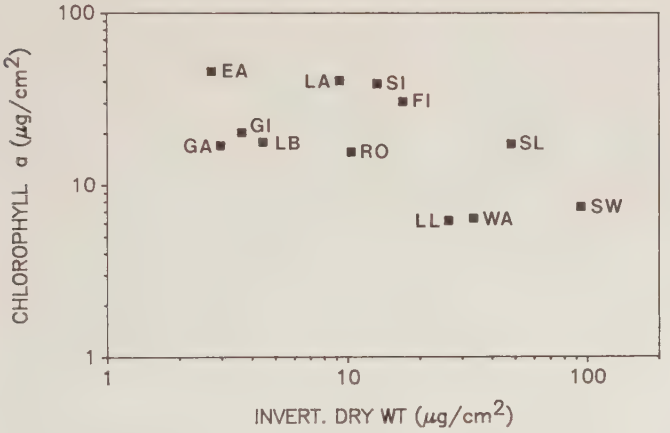

FIG. 6. Relationship between chlorophyll *a* and grazer dry weight on NPC substrata, based upon treatment means ($n = 4$), with associated lake names.

highly concentrated nutrient pools within the substrata far exceeded diffusion that might be predicted from the water column (see Pringle (1987) for a comprehensive discussion of effects on attached algae of nutrient diffusion from the water column versus substratum).

Our results indicated that nutrient limitation by N versus C was generally consistent with predictions based upon water column ratios of DIC/DIN. In contrast, P did not limit growth to the degree predicted. We suggest several mechanisms for the apparently increased access to P within the biofilm.

First, periodic storm events or other disturbances in the littoral zone may greatly modify steady-state diffusion into the mat, for example, both by increasing turbulence at the mat surface (e.g. Riber and Wetzel 1987) and by simultaneously mixing nutrient-rich sediment pore water up into the water column (Peters and Cattaneo 1984). During such nutrient pulses, large quantities of P can be consumed for future use by algal cells (Stevenson and Stoermer 1982). Storage capacity for N appears to be more limited relative to metabolic requirements (Dortsch 1982), and accumulation of excess C is probably very minimal (Goldman and Graham 1981). Periodic disturbances may therefore result in fewer instances of P limitation than might be predicted from steady-state C:N:P ratios in the water column.

Second, work by Pringle (1990) and Mulholland et al. (1991) suggests that phosphatase-mediated acquisition of organic P by benthic algae may be much more important than for phytoplankton owing to the greater potential for accumulation of the enzyme and greater availability of detrital P within the biofilm. Dillon et al. (1990) have also shown that the large majority of detrital seston likely to become incorporated into the developing biofilm on our substrata is actually resuspended sediment that is especially high in P relative to N.

Finally, we have not completely eliminated the possibility of P extraction by the epilithon from the substrata themselves. The release of P from the presoaked clay pots we used to the surrounding water appears to be minimal (Fairchild and Everett 1988). We recognize, however, that clay surfaces may sorb P from the surrounding water, thereby augmenting its supply to colonizing algae (Burkholder and Cuker 1991). Even low levels of contamination may have modified the pool of available P within the biofilm, given its scarcity in the water column.

The apparent control of periphyton photosynthesis by available DIC that we found on the control substrata has also been shown for epiphytes in Lake George, NY (Sheldon and Boylen 1975). Turner et al. (1987) likewise obtained a strong positive correlation between periphyton photosynthesis and available DIC in four softwater lakes and demonstrated enhanced photosynthesis with either $NaHCO_3$ or H_2CO_3 enrichment. At very low alkalinity, depletion of DIC within closed chambers can provide potentially misleading evidence of C-limited growth (Turner et al. 1987), and low P_g values in our study may have been an artifact of DIC depletion in those chambers not receiving diffused bicarbonate.

We found that bicarbonate enrichment also increased standing crop in the most acidic lakes. Dickman (1973) similarly enhanced benthic algal densities with bicarbonate enrichment in an acidic stream emptying Holly Lake, Quebec. Our results are thus consistent with earlier work in suggesting that DIC may limit not only photosynthesis but also standing crop accrual under acidic conditions.

The use of artificial substrata was a necessity in our study, as the naturally occurring rock surfaces varied widely in texture, were virtually absent in several lakes, and were restricted to different depths in others. Algal succession on artificial surfaces, however, is known to influence nutrient limitation of standing crop. In their study of Sycamore Creek, AZ, Grimm and Fisher (1986) showed that added nutrients stimulated the growth of epilithon early in succession but had little effect thereafter. In contrast, nutrient stimulation of epilithon in Douglas Lake, MI (Fairchild and Lowe 1984), was evident only later in succession after standing crops on control substrata had already stabilized. Just how successional state influences nutrient response is likely to depend upon other factors such as current velocity and grazing which also influence standing crop accrual. We chose an incubation time of ~5–6 wk in this study in order to sample biofilms in relatively late stages of succession. We emphasize, however, that our conclusions concerning nutrient response could have been quite different had the substrata been sampled either earlier or much later in time.

Consistently strong nutrient responses, coupled with the inference of relatively weak grazer effects (i.e. the negative correlation between invertebrate biomass and chlorophyll *a* on

NPC substrata), should not be interpreted as evidence that nutrients rather than grazing largely control epilithic algal standing crop in softwater lakes. First, the use of artificial substrata, often placed at some distance from similar natural surfaces, probably permitted more rapid colonization by algae than by invertebrate grazers. The duration of substratum incubation may thus have been insufficient to maximize the numerical response of at least some invertebrata taxa (Fairchild and Lowe 1984). The observed dominance by easily dispersed early instar chironomids (Davies 1976) on the substrata, for example, may not necessarily be representative of natural surfaces in the littoral zone. We also acknowledge that micrograzers, not measured in this study, may have contributed substantially to both algal standing crop losses and the recycling of nutrients within the mat.

We suspect that, at least on the NPC substrata, the degree of grazer control of epilithic algal standing crop was probably lower in the more acidic lakes. The combination of bicarbonate diffusion and intense photosynthesis on the NPC substrata was shown in earlier work to raise pH levels within the mat by as much as 4 units above levels in the water column (to ~10) in another moderately acidic lake in the region (Fairchild et al. 1989). Such a high pH environment may have favored algal taxa not normally encountered by grazers in the more acidic lakes or may have exerted direct toxic effects and thus contributed to reduced grazer colonization of the NPC substrata. We speculate further that the higher chlorophyll *a* levels on NPC substrata in the more acidic lakes are in part the result of pH-mediated reductions in grazing pressure.

In summary, the shift from C limitation of epilithic algal standing crop observed in lakes with low DIC/DIN to N or NP limitation at higher DIC/DIN provides evidence that water column nutrient concentrations may be rather closely linked to growth limitation of the epilithon. The smaller than predicted role of P as a limiting nutrient, if not simply an artifact of nutrient contamination of the substrata, may also indicate the capacity of the epilithon to luxury consume and internally recycle P. The strong attenuation of nutrient availability within the biofilm as standing crop increases, together with removal processes such as grazing, may normally maintain epilithic algal standing crop at low levels despite moderate variation in water column nutrients. Invertebrate grazing appeared less effective, however, in controlling the accumulation of epilithon when nutrients were supplied from a highly concentrated source within the substratum.

Acknowledgements

We thank C. Blundell, M. Martin, M. Mercer, and D. Taylor for field assistance. Most nutrient analyses were supervised by S. Friant. R. Moeller performed the gas chromatographic determinations of DIC. The manuscript was much improved with comments from J. Beneski, T. Frost, L.-A. Hansson, R. Horwitz, M. Potvin, and two anonymous reviewers. S. Jones and the Lacawac Sanctuary provided lodging and technical support of the fieldwork. The study was funded by NSF grant BSR-8604543 and is a contribution to the Pocono Comparative Lakes Program. This paper is dedicated to Dr. Lawrence McManus, longtime Professor of Ecology at Hamilton College, who first got us both interested in aquatic biology.

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Differentiation of *Philonema* spp. (Nematoda; Philometridae) in British Columbia Salmonid Fishes Using DNA Restriction Fragment Length Differences

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Adamson, M. L., D. F. Clease, and L. Margolis. 1992. Differentiation of *Philonema* spp. (Nematoda; Philometridae) in British Columbia salmonid fishes using DNA restriction fragment length differences. Can. J. Fish. Aquat. Sci. 49: 1650–1656.

Restriction fragment length differences were compared in *Philonema* spp. (Nematoda; Philometridae) parasitizing kokanee and sockeye salmon (*Oncorhynchus nerka*) and rainbow and steelhead trout (*Oncorhynchus mykiss*) from various British Columbia localities. DNA extracted from individual worms was digested with various restriction enzymes and the resulting fragments were separated by horizontal gel electrophoresis to reveal bands representing cleavage sites within repetitive DNA sequences. Banding patterns reveal that worms from the two host species represent distinct genetic stocks. Kokanee and sockeye salmon from all localities sampled harboured the same stock of *Philonema*, identified as *P. oncorhynchi*. Worms from rainbow and steelhead trout were clearly distinguished from *P. oncorhynchi* and are assigned to *P. agubernaculum*. *Philonema agubernaculum* from different localities were distinguishable by their banding patterns; material from Pennask Lake resembled that from Babine Lake and could be distinguished from that from O'Connor Lake on Vancouver Island. This similarity may indicate a common postglacial ancestor for Pennask Lake and Babine Lake worms, perhaps by postglacial colonization from the Columbia River system.

Nous avons comparé les différences entre la longueur des fragments de restriction chez *Philomena* spp. (Nematoda; Philometridae) qui parasitent les saumons sockeye et kokanee (*Oncorhynchus nerka*) et les truites arc-en-ciel et steelhead (*Oncorhynchus mykiss*) dans différentes localités de la Colombie-Britannique. L'ADN extrait des vers a été digéré par différents enzymes de restriction et les fragments ainsi produits ont été séparés par électrophorèse horizontale sur gel dans le but de révéler les bandes correspondant aux sites de coupure au sein des séquences répétitives de l'ADN. Les spectres de bandes révèlent que les vers provenant des deux espèces hôtes représentent des stocks génétiques distincts. Les saumons kokanee et sockeye prélevés contenaient le même stock de *Philomena*, à savoir *P. oncorhynchi*. Les vers des truites arc-en-ciel et steelhead ont été clairement distingués de l'espèce *P. oncorhynchi* et ont été caractérisés comme *P. agubernaculum*. Les spécimens de *P. agubernaculum* provenant de différentes localités pouvaient être distingués par leur spectre de bandes; ceux du lac Pennask ressemblaient à ceux du lac Babine et pouvaient être distingués de ceux du lac O'Connor sur l'île de Vancouver. Cette similarité peut indiquer l'existence d'un ancêtre postglaciaire commun pour les vers des lacs Pennask et Babine, peut-être par une colonisation postglaciaire à partir du bassin du fleuve Columbia.

Received September 23, 1991
Accepted February 10, 1992
(JB228)

Reçu le 23 septembre 1991
Accepté le 10 février 1992

Members of the genus *Philonema* are parasites in the body cavity of salmonid fishes in the northern part of North America and Russia. The genus contains nine nominal species, but most are defined in terms of host or zoogeographical differences. Morphological analysis shows considerable overlap (Platzer 1964), and several authors suggested that the nine nominal species may represent as few as two or three valid species (Baylis 1948; Akhmerov 1955; Platzer 1964; Vismanis et al. 1987).

Two species of *Philonema* occur in salmonids in western North America, namely *P. oncorhynchi*, the type species, originally described from sockeye salmon (*Oncorhynchus nerka*) caught in English Bay (Vancouver) (Kuitunen-Ekbaum 1933), and *P. agubernaculum*, originally described from rainbow trout (*Oncorhynchus mykiss*) and other freshwater salmonids in Wyoming (Simon and Simon 1936). Both species have been reported from a variety of British Columbia localities (Margolis 1963, table XXVI; Bailey and Margolis 1987). Although original descriptions suggest that the two species can be distinguished morphologically, later studies cast doubt on this (Baylis 1948; Platzer 1964). The question therefore remains as to how many species of *Philonema* occur in British Columbia.

Curran et al. (1985) demonstrated the feasibility of using restriction fragment length differences (RFLD) in highly repetitive DNA to assess taxonomic relationships among populations and species of nematodes. Closely related species, or populations within a species, share most of the more prominent bands whereas more distantly related taxa share few or no bands. RFLD analysis provides a rapid and relatively easy method to identify *Philonema* spp. in British Columbia salmonids.

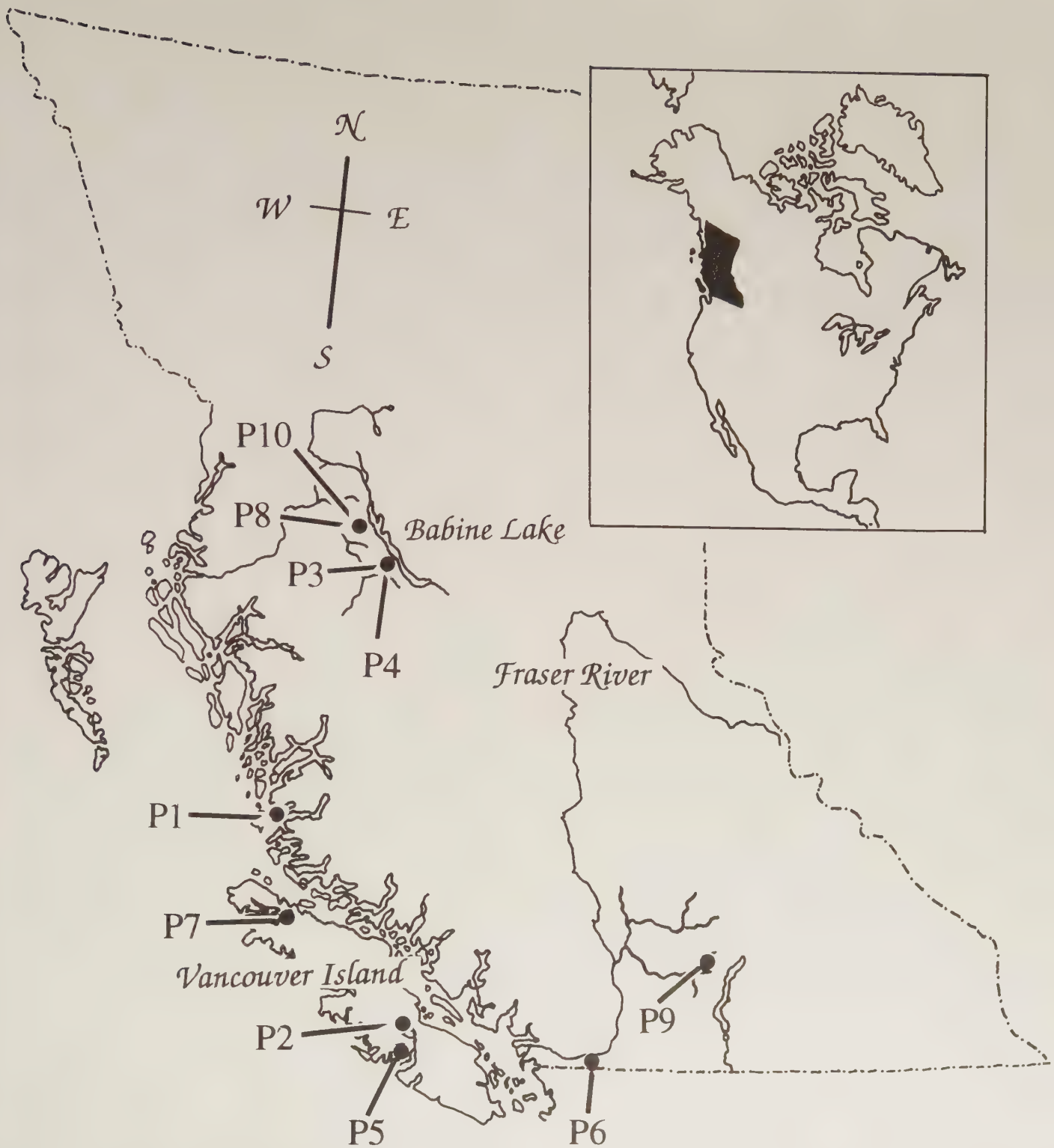

FIG. 1. Map showing hosts and localities from which worm stocks were collected. P1, sockeye salmon from Rivers Inlet; P2, sockeye salmon from Sproat River; P3, sockeye salmon from Pierre Creek (tributary of Babine Lake); P4, kokanee from Pierre Creek; P5, sockeye salmon from Henderson Lake; P6, sockeye salmon from Cultus Lake; P7, steelhead trout from O'Connor Lake; P8, rainbow trout from Fulton River (tributary of Babine Lake); P9, rainbow trout from Pennask Lake; P10, sockeye salmon from Fulton River.

Materials and Methods

Prespawning sockeye salmon and kokanee (a nonanadromous form of sockeye salmon) and rainbow and steelhead trout were collected by seine or dip nets from various British Columbia localities: sockeye salmon from Rivers Inlet, Sproat River, Pierre Creek, Henderson Lake, and Fulton River; kokanee from Pierre Creek; rainbow trout from Fulton River and Pennask Lake; and steelhead smolts from net pens in O'Connor Lake (Fig. 1). Fish were either examined in the field or packed

on ice and shipped to Vancouver for examination.

At least five worms were sampled from each host species and/or collection site. Worms were found lying free in the coelom, entangled in the pyloric caeca, or within the gonads of females. They were removed and placed individually in 1.2-mL cryovials and frozen immediately in liquid nitrogen (−196°C). Worms were later transferred to a freezer at −70°C for storage.

Because of their larger size, only female worms were used for DNA extraction. Worms were removed from cryovials, transfered to 1.5-mL microcentrifuge tubes, and ground under liquid nitrogen using disposable pestles. The resultant powder was digested in 0.7 mL of proteinase K buffer (100 mM Tris (pH 8.0), 50 mM EDTA, 1.0% SDS, and 200 mM NaCl) containing 2 mg of proteinase K (BRL) for 16–20 h at 65°C.

DNA was extracted three times: first with phenol, then with phenol–sevag (chloroform – isoamyl alcohol, 24:1), and finally with sevag. Following extraction, three fifths of a volume of 20% polyethylene glycol 8000 (BDH) in 2.5 M NaCl was added and DNA was precipitated overnight at 4°C. DNA was then pelleted in a microcentrifuge for 15 min, washed twice with cold 70% ethanol, and vacuum dried for 5–10 min before being resuspended in 200 mL of TE (10mM Tris (pH 7.5) and 1 mM EDTA).

The following enzymes were used to digest each sample: Bam H1, Eco R1, Hae III, Hinf I, Hpa I, Msp I, Pst I, Xba I, and Xho I (BRL). Digestion reactions were set up as follows: 2–8 μg of DNA, 2.5 μL of 10× reaction buffer (supplied with the enzyme), 1.0 μL of bovine serum albumen, (2 mg/mL), 10 μL of RNase A (10 mg/mL), and 2.5 μL of restriction enzyme. Reactions were incubated at 37°C for 1–5 h depending on the activity of enzymes and were halted by the addition of 0.5 M EDTA to a final concentration of 10 mM. Loading buffer (15% Ficoll, 0.25% bromophenol blue, and 0.25% xylene cyanol) was added to a final concentration of about 3%.

Samples were loaded onto a 0.7% agarose gel containing 2.5% ethidium bromide, and lambda Hind III fragments were used as size standards. Digests of DNA of *Caenorhabditis elegans* were included for comparative purposes. Gels were run for 18–20 h at 30–40 V to separate restriction fragments. They were illuminated with a UV illuminator and photographed using a Polaroid camera. Banding patterns were traced from negatives.

DNA was transferred overnight to Hybond-N hybridization transfer membranes (Amersham) which were then soaked in 0.4 M NaOH for 20 min, rinsed in 22× SSC (0.02 M sodium citrate and 0.3 M NaCl), and air dried. Membranes were stored at room temperature.

Membranes were probed with a 7.2-kb Eco RI fragment from the pinewood nematode *Bursaphelenchus xylophilus*, including the ribosomal repeat. The probe was nick translated with ^{32}ATP and allowed to hybridize overnight at 62°C in 5× SSPE (5 mM EDTA, 0.06 $NaH_2PO_4 \cdot H_2O$, and 0.7 M NaCl), 0.3% sodium dodecyl sulphate, and 2.5× Denharts (0.05% BSA, 0.05% Ficoll 400, and 0.05% polyvinyl pyrrolidone 40). Membranes were washed once for 5 min and three times for 10 min each in 2× SSPE and 0.2% sodium dodecyl sulphate before being blotted dry and placed in an exposure casette with X-ray film.

Results

Philonema spp. were clearly distinguishable from *C. elegans* by banding patterns produced by all endonucleases examined (Fig. 2–7). Furthermore, only Hae III, Hpa I, and Xho I produced major bands shared by all *Philonema*; the first two enzymes produced 0.4-kb bands whereas the last produced a 3.2-kb band (Fig. 2, 5, and 6).

Most enzymes used separated *Philonema* into two distinct groups: P1–P6 and P10 (collected from sockeye salmon and kokanee) and P7 to P9 (collected from rainbow and steelhead trout).

Worms from *O. nerka* were most easily distinguished from those in *O. mykiss* by banding patterns produced by Hinf I, Hpa I, Xba I, Eco R1, and Hae III. Hinf I produced a series of bands between 1.5 and 4 kb in length in worms from *O. mykiss* that were absent in those from *O. nerka* (Fig. 2). Similarly, Hpa I produced a series of prominent bands in the 1- to 2-kb range in worms from *O. mykiss* that were absent in those from *O. nerka* (Fig. 5). A series of prominent bands between 1 and 2.3 kb in length were present in Xba I digests of worms from *O. nerka* but absent in worms from *O. mykiss* (Fig. 4). Eco R1 produced only a single prominent band about 15 kb in length in worms from *O. mykiss* and two bands, one about 4 kb the other about 5 kb in length, in worms from *O. nerka* (Fig. 7). Finally, worms from *O. mykiss* showed a prominent Hae III band 1.4 kb in length not shown by those from *O. nerka* but lacked a band 0.5 kb in length shown by these latter worms (Fig. 6).

Worms collected from *O. mykiss* in different geographic localities differed with respect to patterns produced by Bam HI, Hae III, and Hinf I. Bam HI produced a 2.1-kb band that was present in worms from Fulton River and Pennask Lake and absent in those from O'Connor Lake. Two bands, one 3.1 kb and the other 2.8 kb, produced with Hinf I digestion were present in worms from Fulton River and Pennask Lake and absent in those from O'Connor Lake, while a 2.6-kb band present in worms from O'Connor Lake was absent in worms from other localities (Fig. 2). Hae III digestion produced different patterns in worms from *O. mykiss* from each of the three localities. Worms from O'Connor Lake showed two bands, one 3.5 kb and the other 1.1 kb, not present in those from other localities. Worms from Fulton River *O. mykiss* had a 2.4-kb Hae III band not present in other worms and lacked a 2.8-kb band that was present in worms from *O. mykiss* from Pennask and O'Connor lakes (Fig. 6).

Worms from different populations of *O. nerka* differed little. Individual differences were observed among worms with respect to banding patterns created by Bam HI, Hinf I, and Hpa I, but these were not restricted to single populations. Bam HI digestion produced a 2.6-kb band that was present in Cultus Lake worms, in three of five Sproat Lake worms, in one of five worms from each of Henderson Lake and Pierre Creek, and in much fainter form in worms from Fulton River. Similarly, worms were polymorphic for two bands, 1.0 and 0.9 kb, produced by Hinf I digestion (Fig. 2). The 0.9-kb band was the more prominent band in worms from Cultus Lake, in three of five worms from Sproat River, and in one worm from each of Henderson Lake and Pierre Creek. In other worms collected from Henderson Lake, Pierre Creek, and Sproat River and in all worms from Rivers Inlet, the 1.0-kb band was of greater intensity. Finally, a 1.9-kb band produced by Hpa I digestion was present in Cultus Lake worms, in three of five Sproat River worms, and in one worm from Pierre Creek (Fig. 5); individual variation in Hpa I banding pattern was not assessed in material from sockeye salmon from Henderson Lake and Fulton River.

Results of hybridization with the ribosomal probe from *B. xylophilus* are summarized in Fig. 8. Southern blots were prepared of DNA digested with Hae III, Hpa I, Hinf I, Xho I,

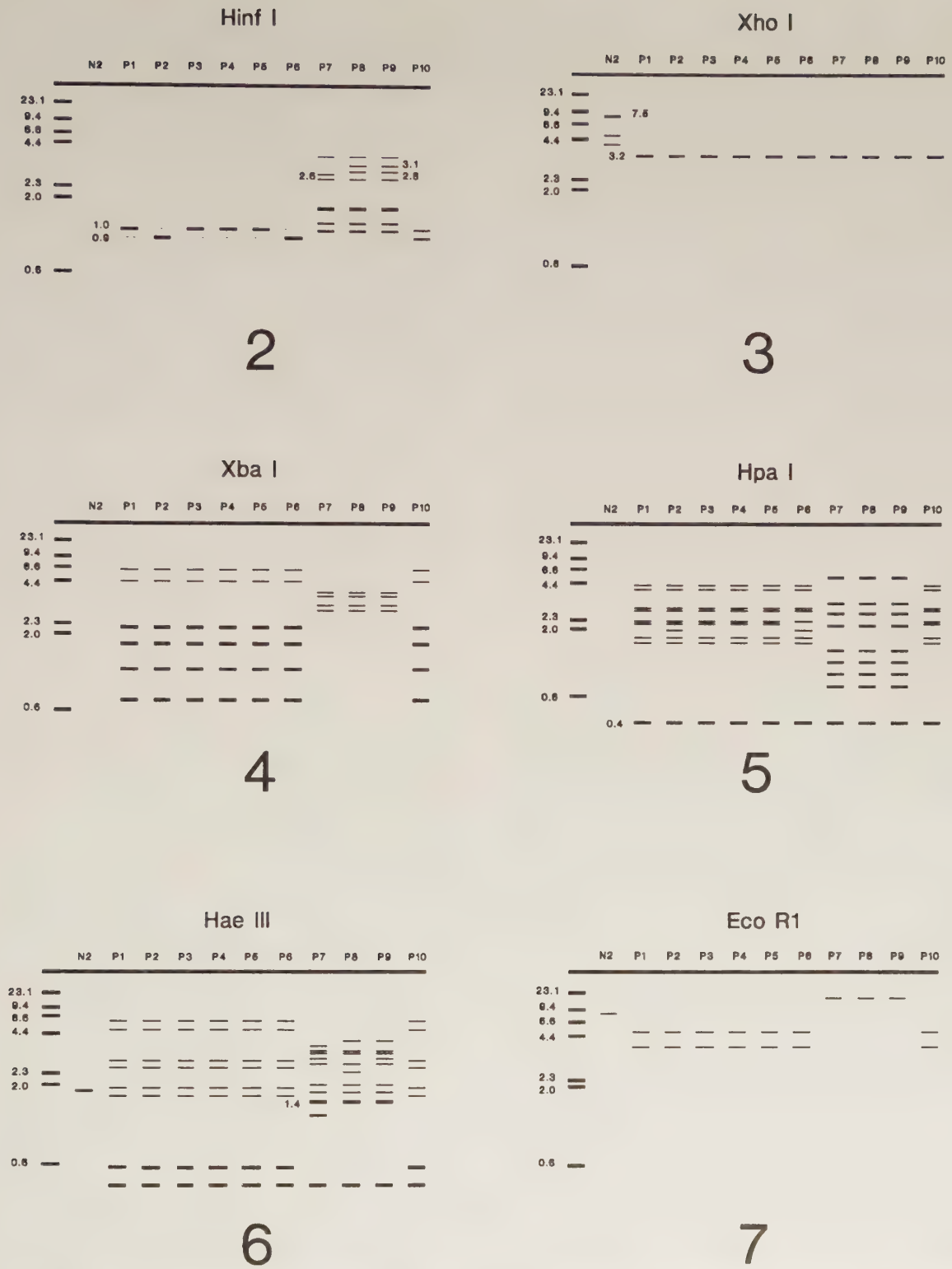

FIG. 2–7. Summary of major bands produced by restriction of genomic DNA of *Philonema* spp. from various hosts and localities. In all figures, the lane on the far left is lambda DNA cut with Hind III, used as a size standard. See Fig. 1 for stock origins. Note that only three of the endonucleases (Xho I, Hpa I, and Hae III) reveal bands shared by all isolates of *Philonema* and that isolates from trout (P7, P8, and P9) are clearly distinguished from those in sockeye salmon (P1–P6 and P10). All digests were replicated on at least five worms from each collection site.

and Bam HI. In all but Bam HI, material from trout was compared with that taken from salmon. No differences were observed in the hybridization pattern between worm populations. Hae III digestion produced four fragments that hybridized with the probe; the 1.4-kb fragment does not appear to be homologous to the fragment of similar size that differentiates salmon worms from those in trout (see Fig. 6).

The most parsimonious arrangement of shared bands described herein is depicted in Fig. 9. Worms from *O. nerka* are clearly separated from those in *O. mykiss*. Worms from Ful-

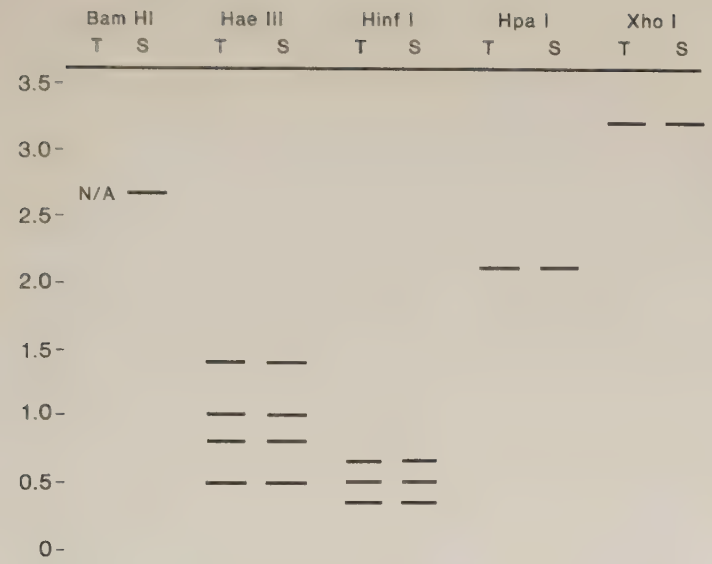

FIG. 8. Scheme summarizing results of hybridization of restricted DNA from worms collected from trout (T) and salmon (S) with a ribosomal probe pBx 2 taken from *Bursaphelenchus xylophilus* (Tylenchida; Nematoda). No differences were observed between worms from the two host sources.

ton River (P8) and Pennask Lake (P9) populations of *O. mykiss* are more similar to each other than they are to those recovered from O'Connor Lake (P7) hosts; Hae III produced a homoplasious band 2.8 kb in length in P9 and P7. Worms from the various sources of *O. nerka* were similar.

Discussion

This study uses banding patterns produced by restriction endonucleases on total DNA extractions of worms to assess phylogenetic relationships. Restriction endonucleases cut DNA at specific sites as recognized by the base composition at that site; the fragments of DNA are then separated by size on an agarose gel and visualized by ethidium bromide staining. Bands of similar size produced by the same enzyme are assumed to be homologous. Enzymes may produce similarly sized fragments that differ strongly in base composition, but in order to produce a band, the enzyme must produce a strong overrepresentation of fragments of a particular size. Thus, enzymes do not typically produce bands of the same size in unrelated stretches of DNA and the assumption of homology is generally valid. Hae III produced what are presumably nonhomologous 2.8-kb bands in worms from Lake O'Connor and Pennask Lake in the present study, but otherwise, there was strong congruence of banding patterns.

DNA probes can be used to produce banding patterns based on known, or at least homologous, DNA fragments. In the present study, we present limited data using a ribosomal probe. Although we did not detect differences, we were only able to examine patterns produced by four enzymes. We would expect differences to be found if more enzymes were examined because the ribosomal fragment includes long stretches of untranscribed DNA that are subject to rapid change.

Species of *Philonema* can be divided into two groups based on host biology. *Philonema oncorhynchi, P. ochotense* Fujita, 1937, *P. kondai* Fujita, 1939, *P. tenuicauda* Fujita, 1939, and *P. elongata* Fujita, 1940 occur in anadromous salmonids of the genus *Oncorhynchus*. These hosts typically undergo long ocean migrations and spawn only once in their life. *Philonema agu-*

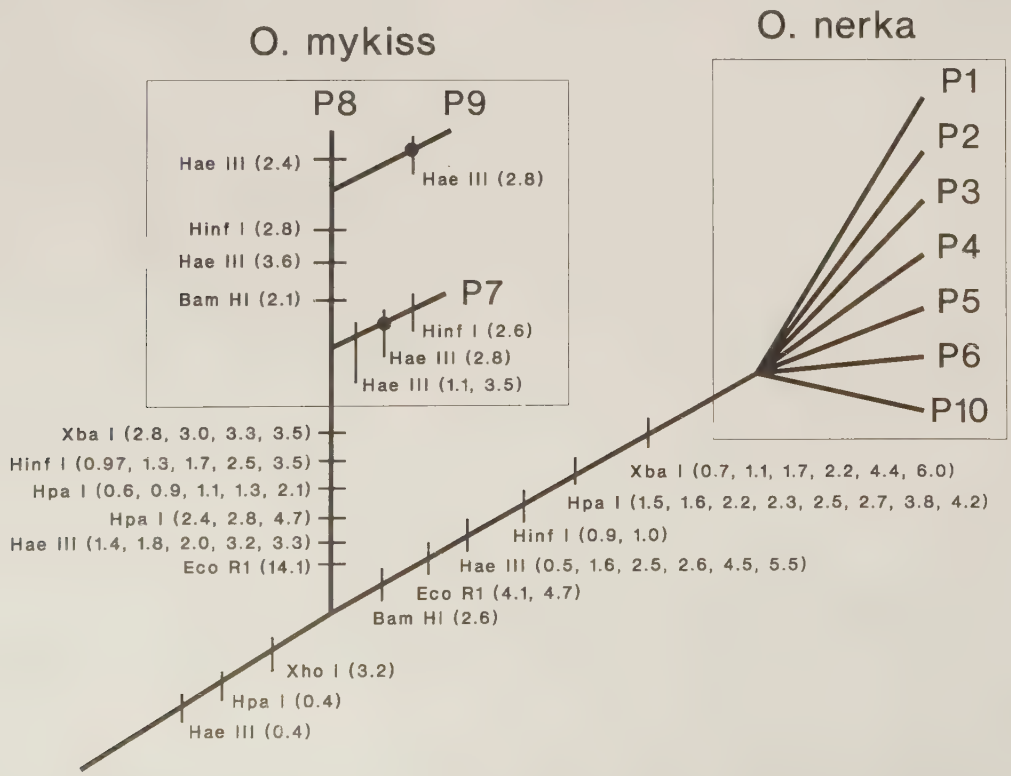

FIG. 9. Cladogram summarizing the most parsimonious mapping of shared bands produced by restriction of genomic DNA in *Philonema* spp. from various localities. See Fig. 1 for stock origins. The single homoplasious band was one 2.8 kb in size produced by Hae III digestion and indicated with a closed circle.

bernaculum, *P. salvelini* Fujita, 1939, *P. salvelini* Richardson, 1937, and *P. sibirica* (Bauer, 1946) occur in nonanadromous salmonids of diverse genera (*Coregonus*, *Oncorhynchus*, *Prosopium*, *Salmo*, and *Salvelinus*) that breed several times in their life.

Philonema spp. are morphologically very similar, and there is doubt as to the validity of many species. Baylis (1948) noted that *P. agubernaculum* may be identical with *P. oncorhynchi*, and Akhmerov (1955) considered *P. agubernaculum*, *P. elongata*, and *P. sibirica* as synonyms of *P. oncorhynchi*. Vismanis et al. (1987) recognized only *P. oncorhynchi* and *P. sibirica* from Russia and listed *P. ochotense*, *P. kondai*, *P. tenuicauda*, *P. elongata*, and *P. salvelini* Fujita as synonyms of *P. oncorhynchi*.

On the basis of RFLD analysis, *Philonema* from *Oncorhynchus* spp. in British Columbia can be divided into two distinct groups, corresponding to the different hosts in which they were found. Worms collected from *O. mykiss* appear to be genetically distinct from those in *O. nerka*. This applies to worms collected where the two hosts are sympatric (e.g. Babine Lake). We conclude that worms in these host species represent distinct species. Material from *O. nerka* is assigned to *P. oncorhynchi* because this species was originally described from sockeye salmon in British Columbia. We assign material from *O. mykiss* to *P. agubernaculum*, originally described from *O. mykiss*, *Salvelinus fontinalis*, and *Prosopium williamsoni* in Wyoming and later reported from *O. mykiss*. *Oncorhynchus clarki*, and *S. fontinalis* in Idaho (Chacko 1975).

Material collected from kokanee salmon was similar to that collected from sockeye salmon and can therefore be assigned to *P. oncorhynchi*. This is not surprising because kokanee represent a self-sustaining freshwater offshoot of anadromous sockeye salmon populations (Scott and Crossman 1973; Foote et al. 1989). Like sockeye salmon, kokanee spawn once in their life, usually in their fourth year. Kokanee and sockeye salmon sharing the same nursery lake presumably share the same population of *P. oncorhynchi*.

Transmission in *Philonema* spp. involves a freshwater copepod intermediate host in which development from the first to third larval stage occurs. Fish are infected when they ingest infected copepods. In British Columbia, *P. oncorhynchi* and *P. agubernaculum* probably use the freshwater copepod *Cyclops bicuspidatus* as an intermediate host (Platzer and Adams 1967; Ko and Adams 1969). Dependence upon a pelagic lake-dwelling intermediate host presumably explains why sockeye are the only anadromous *Oncorhynchus* sp. commonly infected by *Philonema*. With few exceptions, juveniles of sockeye salmon stocks spend one or more years in a nursery lake, where they acquire *Philonema* infections before migrating out to sea. Other anadromous species of *Oncorhynchus* generally do not include a lake phase in their life histories (Groot and Margolis 1991).

Steelhead trout, whose smolts occur in streams and rivers, are not commonly infected naturally by *Philonema* spp. (L. Margolis, unpubl. data). Infected steelhead trout studied herein were being reared in net pens in a lake and presumably the source of their infections were rainbow trout resident in the lake. Banding patterns of their DNA resembled that of worms from wild-caught rainbow trout from Pennask Lake and Fulton River.

Philonema agubernaculum and *P. oncorhynchi* probably use the same species of intermediate host, yet cross-infections with mature worms of the two species seem to be nonexistent or rare even where the two species occur in sympatry. Sockeye salmon spawn in late summer and fall, whereas rainbow trout spawn in spring. Because gravid female worms leave the hosts as the latter spawn, *P. oncorhynchi* probably infects copepod intermediate hosts in late summer and fall whereas *P. agubernaculum* would infect copepods in spring. Nevertheless, there are sockeye salmon and rainbow trout feeding on copepods throughout the year and the factors that isolate the two species of *Philonema* where they occur sympatrically would appear to be unrelated to this seasonal difference in infection of intermediate hosts.

Bashirullah (1966, 1983) conducted reciprocal cross-transmission studies and demonstrated that both worms could survive and develop in the other's host. He argued that hybridization between the two species was prevented because of life history differences. According to him, *P. oncorhynchi* develops slowly and is dependent for its maturation upon elevated hormone levels of a reproductively mature host. *Philonema agubernaculum*, however, was thought to develop directly to adulthood independent of host hormonal status. Bashirullah (1966, 1983) considered that *P. agubernaculum* was unable to survive the long migration of sockeye salmon.

Unpublished data (L. Margolis) from Fraser River sockeye salmon indicate that adult fish of most stocks are free of *P. oncorhynchi*, yet smolts from these stocks may have a high prevalence (although low intensity) of infection with *Philonema* larvae. For this reason, Bailey and Margolis (1987) identified the larvae in sockeye salmon smolts from these stocks as *P. agubernaculum*. Also, fully gravid *P. agubernaculum* are found in juvenile steelhead trout reared in net pens in O'Connor Lake, within less than a year of acquiring infections (L. Margolis and T. E. McDonald, Pacific Biological Station, Nanaimo, B.C., unpubl. data). These observations tend to support Bashirullah's (1966) explanation of the mechanism that maintains the reproductive isolation of *P. oncorhynchi* and *P. agubernaculum*.

Philonema agubernaculum showed banding patterns specific to geographic localities from which they were collected. This is presumably because the worms, like their trout hosts, are restricted to specific drainage basins. Pennask Lake and Fulton River populations were nearly identical, distinguished only by two HAE III bands, whereas O'Connor Lake worms differed from the two mainland populations by aspects of banding patterns produced by three enzymes (Bam HI, Hae III, and Hinf I).

Melting and subsequent retreat of glaciers during the last glaciation resulted in formation of large lakes and streams (Briggs 1986) which facilitated reinvasion of freshwater fish from areas of refugia in the north and south. The Fraser River system was principally recolonized from the Columbia River via a succession of glacial lakes in the Okanagan Valley. The Skeena River (which drains Babine Lake via Babine River) has a fish fauna similar to that of the Columbia River, suggesting a previous connection via the Fraser, probably while the Skeena was blocked and its tributaries flowed east into the Fraser (McPhail and Lindsey 1986). Thus the relationship between Pennask Lake and Fulton River *Philonema* populations may reflect their common origin from the Columbia River system. On the contrary, *P. agubernaculum* from O'Connor Lake may have diverged much earlier following an early postglacial migration of its rainbow trout host from the mainland across the Strait of Georgia. Alternatively, O'Connor Lake worms may represent a population that survived in a central coast refugia during the Pleistocene glaciation. There is increasing evidence, most of it botanical, that areas in the Queen Charlotte Islands and northern Vancouver Island remained unglaciated during the most recent

Pleistocene glaciation (McPhail and Lindsey 1986; Ogilvie 1989). If *P. agubernaculum* from O'Connor Lake represents a population that survived in this refugium, it should be similar to material from the Queen Charlotte Islands. *Philonema agubernaculum* from southern Vancouver Island may be more similar to populations on the mainland because it was probably colonized from this area following the glacial retreat. These speculations remain to be tested by study of *Philonema* from freshwater-resident salmonids in the Queen Charlotte Islands, southern Vancouver Island, and the southern coastal mainland of British Columbia.

Fixed differences were not observed among populations of *P. oncorhynchi*. Sockeye salmon tend to home to their natal stream, and if homing were perfect, gene flow among worm populations would be restricted just as it appears to have been in *P. agubernaculum*. Quinn et al. (1987), using protein electrophoresis and parasite data, estimated that sockeye salmon may stray less than 1% of the time. However, even this small amount of straying may be enough to prevent population divergence within *P. oncorhynchi* because each female worm releases thousands of larvae.

The fact that transmission of *Philonema* spp. occurs in freshwater and that the occurrence of *Philonema* in freshwater salmonids (including landlocked *Salmo salar*) is widespread suggests that the genus arose in freshwater hosts. Pacific salmon of the genus *Oncorhynchus* are a recent lineage, 2–3 million yr old (Thomas et al. 1986), and sockeye salmon, the primary host of *P. oncorhynchi*, diverged from this lineage about 2 million yr ago. *Philonema oncorhynchi* may have evolved from an ancestor found in nonanadromous salmonids in response to a change in the host life history. However, *P. oncorhynchi* does not occur throughout its primary host's range; it is absent from the upper Fraser River and the Columbia River drainage (Margolis 1963, table XXVI; Bailey and Margolis 1987). This suggests that the species may have arisen as a capture (speciation involving a host shift) sometime after sockeye salmon had diverged from other *Oncorhynchus* species. This may have occurred on the Asian or Alaskan coast during the Pleistocene glaciation with subsequent colonisation of the British Columbia coast occurring when sockeye salmon populations reinvaded from areas of refugia in Beringia (Lindsey and McPhail 1986).

Acknowledgments

The authors gratefully acknowledge the help of Stu Barnetson of the Department of Fisheries and Oceans' Fulton River Salmon Enhancement Project for assistance with collection of fish and of Karen Bechenbach and Dr. J. M. Webster (Department of Biology, Simon Fraser University) for supplying the ribosomal probe. Robert Carveth (Department of Zoology, University of British Columbia) commented on various drafts of the manuscript. This study was supported in part by a grant from NSERC (UBC-80875) to M.L.A.

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Use of a Binomial–Normal Functional Model to Demonstrate that the Sex and Age Composition of Pacific Hake (*Merluccius productus*) Aggregations Affects Estimates of Mean Lengths-at-Age

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Smith, B. D., M. W. Saunders, and G. A. McFarlane. 1992. Use of a binomial–normal functional model to demonstrate that the sex and age composition of Pacific hake (*Merluccius productus*) aggregations affects estimates of mean lengths-at-age. Can. J. Fish. Aquat. Sci. 49: 1657–1669.

We present statistical evidence that estimates of mean length-at-age from a single aggregation of Pacific hake (*Merluccius productus*) are unlikely to represent those for the entire local population because fish length varies with age and sex, and fish aggregations are probably structured by fish size. We present series of simple linear relationships between estimates of mean length-at-age from hake sample sets and both the proportion of males and the proportions-at-age in those sample sets. To determine these relationships, we developed a likelihood-based model which incorporated model and sampling uncertainty in estimates of mean lengths-at-age, and sampling uncertainty in the estimated proportions of males or proportions-at-age. The results show that if fish length varies with sex or age, then the mean lengths-at-age estimated from a single sample set will be predictably, although perhaps modestly, influenced by the age and sex composition of the sample set. Thus, estimates of population mean lengths-at-age, and other sex–age–size statistics of fish that form aggregations, should be based on the premise that an aggregation of fish, not an individual fish, is the basic sampling unit.

Nous présentons des preuves statistiques selon lesquelles les estimations de la taille moyenne à l'âge adulte effectuées à partir d'un seul regroupement de merlus du Pacifique (*Merluccius productus*) ne représentent vraisemblablement pas celles de la totalité de la population locale, car la taille des poissons varie selon l'âge et le sexe, et la structure des regroupements varie probablement en fonction de leur taille. Nous présentons une série de relations linéaires simples entre les estimations de la taille moyenne à l'âge adulte dans des ensembles d'échantillons de merlus et la proportion de mâles et la proportion d'adultes dans ces ensembles d'échantillons. Pour caractériser ces relations, nous avons élaboré un modèle basé sur la vraisemblance qui incorpore l'incertitude du modèle et de l'échantillonnage dans des estimations des tailles moyennes à l'âge adulte, et incorpore l'incertitude de l'échantillonnage dans les proportions estimées de mâles ou d'adultes. Les résultats montrent que, si la taille des poissons varie avec le sexe ou l'âge, les tailles moyennes à l'âge adulte estimées à partir d'un seul ensemble d'échantillons seront de manière prévisible, quoique peut-être modeste, influencées par la composition de l'ensemble d'échantillons sur le plan de l'âge et du sexe. Ainsi, les estimations des tailles moyennes à l'âge adulte de la population et les autres statistiques sexe–âge–taille sur les poissons qui forment des regroupements devraient considérer le regroupement de poissons et non le poisson comme unité fondamentale d'échantillonnage.

Received August 27, 1991
Accepted February 11, 1992
(JB201)

Reçu le 27 août 1991
Accepté le 11 février 1992

Pacific hake (*Merluccius productus*) is an abundant and commercially important pelagic fish inhabiting the coastal waters off western North America (Bailey et al. 1982; Beamish and McFarlane 1985). Populations of hake have been identified in the Strait of Georgia (McFarlane and Beamish 1985), Puget Sound (Pedersen 1985), and coastal inlets of British Columbia (Beamish and McFarlane 1985; Shaw et al. 1985, 1989), but most hake belong to a large offshore population. This offshore population undergoes dramatic seasonal migrations from winter spawning locations off California and northern Mexico (Bailey et al. 1982; Bailey and Francis 1985) to summer feeding locations off southwestern Vancouver Island, British Columbia (Beamish 1981; Beamish et al. 1982; Barner et al. 1984; Shaw et al. 1985, 1989), where oceanographic conditions promote high productivity (Thomson and Ware 1988; Ware and McFarlane 1989). A well-documented phenomenon of offshore Pacific hake population dynamics is a trend of increasing mean lengths-at-age of males and females with increasing latitude during summer (Francis 1983; Beamish and McFarlane 1985; Nelson and Dark 1985) when the hake have segregated by length probably as a result of length-dependent migration rates (Smith et al. 1992).

As anticipated, this phenomenon was apparent as an increasing trend in sample mean lengths for males and females in a series of 11 trawl samples collected consecutively along a generally south to north transect along the west coast of Vancouver Island during August 1989 (Fig. 1). However, in addition to the trend of increasing mean lengths of males and females, we also noticed that the pattern of variation in mean lengths appeared, in general, to mirror the sex ratio of the samples collected (Fig. 1). In particular we noticed that, notwithstanding the trend, the proportion of males in the sample tended to vary inversely with the mean lengths of both males and females in the sample. Female hake tend to be larger than male hake at a given age older than 3 yr; thus, our observations led us to hypothesize that the observed relationship between the sample

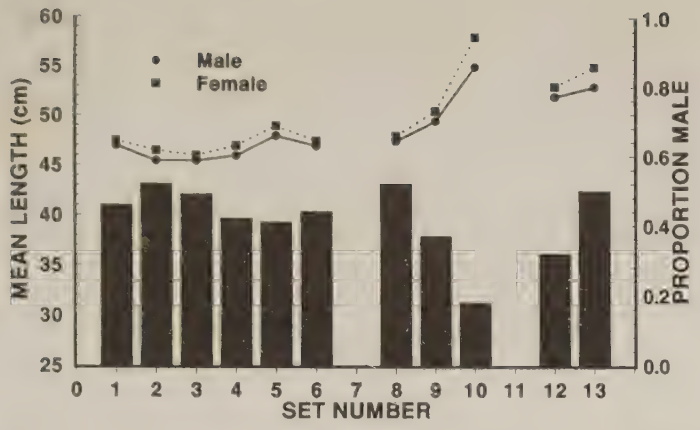


FIG. 1. Comparison of mean lengths of male and female offshore Pacific hake in 11 sample sets collected off Vancouver Island in summer 1989 (points) with the proportion of males (bars) in the sample sets. Sample sets 1 to 13 were collected consecutively along a generally south to north transect from about 49°N to 51.4°N. Note that variation in the trend of increased mean length with latitude tends to mirror the sex composition of the sample sets. Sample sets 7 and 11 did not capture hake.

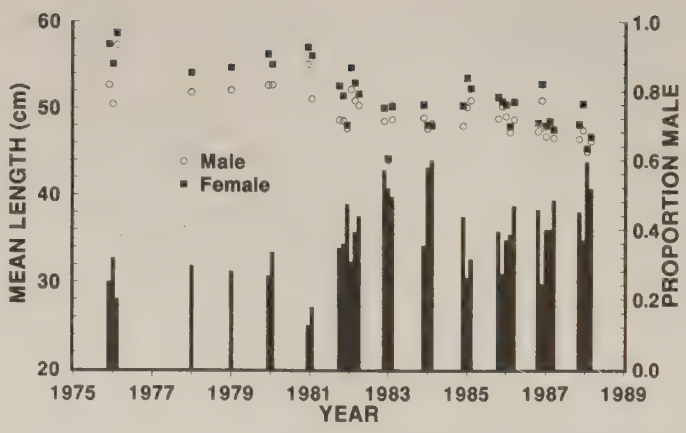


FIG. 2. Comparison of mean lengths of male and female offshore Pacific hake in 38 sample sets collected off southwestern Vancouver Island from 1976 until 1988 (points) with the proportion of males (bars) in the sample sets. This plot demonstrates the feature of decreased hake mean length over time (Smith et al. 1990). Note that variation in the trend of decreased mean length over time, and among sample sets of a particular year, tends to mirror the sex composition of the sample sets.

sex ratio and the mean lengths of males and females in the sample is an indication that aggregations of hake are structured by fish size.

If aggregations of hake are organized by size, then in a statistical sense there is a tendency for individual fish within an aggregation to have an affinity to some size characteristic of the aggregation, say the mean size. If individuals join an aggregation based on their size, then those aggregations will have sex and age compositions that do not represent the entire population of interest. Consequently, mean lengths-at-age or mean lengths of males or females from a single aggregation will not represent those within other aggregations or the entire population of interest. For example, if by chance a single trawl set happened to sample an aggregation of predominantly larger, and consequently older, hake, then males and females of younger ages within that aggregation would tend to be larger than the average size of males and females of those younger ages in the entire population of interest. Similarly, with female hake tending to be larger than male hake, samples containing larger hake will tend to display a sex ratio in favor of females, while samples containing smaller hake will tend to display a sex ratio in favor of males (e.g. Fig. 1).

Complex schooling or aggregating behavior in fish populations is a well-known phenomenon that has been studied extensively by animal behaviorists. A general knowledge that schooling results from complex interactions among fish means that statistical analyses of fish sex–age–size relationships are usually interpreted cautiously, since biases can result from such nonrandom behavior. However, despite a strong conceptual understanding that biases can result from fish aggregation behavior, the fisheries literature is devoid of statistical analyses of the potential effects of such behavior on the establishment of sex–age–size relationships for a population of fish. In this paper we present such an analysis for offshore Pacific hake after we reduce other possible sources of such statistical bias which can result from the migratory behavior of offshore Pacific hake.

An essential component of our analyses is the introduction of a binomial–normal functional model to fisheries literature. This model facilitates analysis of the functional relationship between estimates of mean lengths-at-age and estimates of either the proportion of males or proportion-at-age. Although an ordinary regression model might appear appropriate for our data, with the independent variable being either the measured proportion of males or proportion-at-age, large binomial sampling error falsifies the critical assumption of ordinary regression that the independent variable be measured without error. We demonstrate that ignoring this sampling error when binomial sample sizes are small can lead to a misleading interpretation of the relationship betwen mean lengths-at-age and sex or age composition. The binomial–normal functional model we introduce can be widely applied to problems where a simple linear relationship between binomially and normally distributed bivariate data seems appropriate.

Sex–Age–Length Data

Offshore Pacific hake have been collected along the continental shelf break (Thomson et al. 1989) off southern Vancouver Island between 48°20′N and 49°N during summer annually since 1976, 1977 being the only year when no samples were collected. Sex–age–length data were obtained for random samples of hake taken from 38 midwater trawl catches by research vessels (Beamish 1981; Beamish et al. 1982; Barner et al. 1984; Shaw et al. 1985, 1987, 1989) or foreign commercial vessels (Davenport 1985). In this paper a sample set refers to a subsample of hake from a single trawl sample which is presumed to sample a single aggregation of hake. Individual hake within a sample set were assessed to determine their sex and fork length to the nearest millimetre, and then the largest pair of otoliths was collected for age determination according to the procedures of Beamish (1979). There were generally few hake older than 14 yr in our samples and they showed little interage variation in mean length-at-age, so as in Smith et al. (1990, 1992), we combine all hake 14 yr and older into a $14^{>}$ age-class. There were few hake younger than 3 yr in our samples; therefore, we consider only hake 3 to $14^{>}$ yr old.

Our sex–age–length data were summarized to yield sample mean lengths-at-age (y_{hj}) and their standard errors (v_{hj}) for both males and females (see Table 1 for symbol definitions). Thus for both males and females, our complete set of observed data

TABLE 1. Definitions and units for symbols representing variables used in the binomial–normal functional model.

Symbol	Units	Definition
h	—	Index for sample sets $h = 1, \ldots, m$
i	—	Index for age of influence $i = 3, \ldots, 14^{>}$
j	—	Index for age influenced $j = 3, \ldots, 14^{>}$
y_{hj}	cm	Observed mean length-at-age j in set h
$\bar{y}_{\cdot j}$	cm	Sample mean of y_{hj} over sets $h = 1, \ldots, m$
Y_{hj}	cm	Expected mean length-at-age j in set h for each P_{hj}
Y_{hij}	cm	Expected mean length-at-age j in set h for each P_{hij}
$\bar{Y}_{\cdot j}$	cm	Mean of Y_{hj} or Y_{hij} (for any i) over sets $h = 1, \ldots, m$
λ_j^2	cm^2	Model error variance
υ_{hj}^2	cm^2	Sampling error variance about Y_{hj}
σ_{hj}^2	cm^2	Total variance ($\lambda_j^2 + \upsilon_{hj}^2$)
K	yr^{-1}	Annual rate of change in $\bar{Y}_{\cdot j}$, λ_j^2, β_j, and β_{ij}
n_h	—	Number of hake collected in set h
s_h	—	Number of male hake collected in set h
r_{hi}	—	Number of hake of age i collected in set h
p_h	Dimensionless	Measured proportion of males in set h (s_h/n_h)
P_{hj}	Dimensionless	Proportion of males in set h as estimated by the "sex composition" model for each age j
$\bar{P}_{\cdot}$	Dimensionless	Mean of P_{hj} (for any j) over sets $h = 1, \ldots, m$
p_{hi}	Dimensionless	Measured proportion of hake of age i in set h (r_{hi}/n_h)
P_{hij}	Dimensionless	Proportion of hake of age i in set h as estimated by the "age composition" model for each age j
$\bar{P}_{\cdot i}$	Dimensionless	Mean of P_{hij} (for any j) over sets $h = 1, \ldots, m$
ϕ_{hj}^2	cm^2	Variance ($n_h P_{hj}[1 - P_{hj}]$) about P_{hj}
ϕ_{hij}^2	cm^2	Variance ($n_h P_{hij}[1 - P_{hij}]$) about P_{hij}
α_j	cm	Intercept Y_{hj} at $P_{hj} = 0$
β_j	cm	Rate of change in $Y_{\cdot j}$ over the domain (0 to 1) of p.
β_{ij}	cm	Rate of change in $Y_{\cdot j}$ over the domain (0 to 1) of $p_{\cdot i}$
D_j	cm	$\bar{Y}_{\cdot j} - \bar{P}_{\cdot}$
D_{ij}	cm	$\bar{Y}_{\cdot j} - \bar{P}_{\cdot i}$
L	Dimensionless	Likelihood
$l(\Theta.)$	Dimensionless	Negative log-likelihood for the parameter vector Θ.
N	Dimensionless	Random normal deviate of Y_{hj}
B	Dimensionless	Random binomial deviate of P_{hj} or P_{hij}

consists of 38 mean lengths-at-ages for each of ages 3 to $14^{>}$ (y_{hj}), 38 estimates of the standard error of y_{hj} (υ_{hj}) for each of ages 3 to $14^{>}$ if y_{hj} is determined from a sample size (r_{hi}) of two or more individuals, 38 counts of the number of hake (n_h) in a sample set, 38 counts of the number of males (s_h) in a sample set, and 38 counts of the number of hake of ages 3 to $14^{>}$ (r_{hi}) within each sample set.

Our purpose in this paper is to show that a relationship exists between the sex or age composition of a hake sample set and mean lengths-at-age estimated from that sample set. To do this objectively we must first eliminate other possible causes of this relationship. The only other possible cause apparent to us from our data is the coincidence of a decline in mean lengths of sample sets from 1976 until 1988 with an increase in the proportion of males in those sample sets (Fig. 2). This decline has been attributed to selective fishing of larger, and predominantly female, hake in Canadian waters which have been partially segregated from smaller, and predominantly male, hake found further south (Smith et al. 1990). This segregation can be explained by length-dependent migration rates from winter spawning locations in waters off California or northern Mexico (Smith et al. 1992), which affords the larger hake a better opportunity to reach Canadian waters. Female hake tend to be larger than male hake for ages older than 3 yr; thus, selective fishing of large fish will tend to alter the population sex ratio in favor of males.

To remove the time series relationship between the proportion of males in a sample set and mean lengths-at-age estimated from the sample, we used only data from sample sets collected after 1981. Study of Fig. 2 indicates that excluding data for years prior to 1982 removes the relationship over time between the population sex ratio and the mean lengths of the sample sets. Note also in Fig. 2 that variation in mean lengths among sample sets of a particular year tends to mirror the sex composition. Exclusion of the nine sample sets collected prior to 1982 leaves data from $m = 29$ sample sets for formal analysis of the relationship between the proportion of males or proportion-at-age in a sample set and mean lengths-at-age estimated from that sample set. We assume in the analyses that follow that these 29 sample sets belong to a population of interest that we define as those hake feeding off southern Vancouver Island during summer regardless of the year of the sample.

Binomial–Normal Functional Model

We use generally familiar notation in the description of our binomial–normal functional model, but the specific model which follows uses some notation unique to our sex–age–length data (see Table 1). In particular we use the subscript j to index age. Readers interested only in a generic definition of the binomial–normal functional model need simply ignore the subscript j, while retaining the subscript h to index data records, in this section, in Appendices A, B, and C, Tables 2 and 3, and Fig. 3.

A simple linear relationship between the variable Y_{hj} and P_{hj} is generally represented by the familiar model.

$$(1) \qquad Y_{hj} = \alpha_j + \beta_j P_{hj} + \lambda_j N_h$$

where α_j represents the intercept, β_j the slope and λ_j^2 the model error variance. The symbol N_h represents a standard normal deviate with mean 0 and variance 1. In our application, Y_{hj} represents the expected mean length-at-age j for sample set h, and P_{hj} represents the expected proportion of males of age j in a sample set h.

We found that the parameters of this model had more direct statistical interpretation if Eq. 1 was reparameterized as

$$(2) \qquad Y_{hj} = \beta_j P_{hj} + (1 - \beta_j)\bar{P}_{\cdot} + D_j + \lambda_j N_h$$

(Schnute et al. 1990) where $\bar{P}_{\cdot}$ represents the mean of the P_{hj}'s (for any j) and

$$(3) \qquad D_j = \bar{Y}_{\cdot j} - \bar{P}_{\cdot}$$

with $\bar{Y}_{\cdot j}$ representing the mean of the Y_{hj}'s. A value for α_j can be obtained from the parameters of Eq. 2 using

$$(4) \qquad \alpha_j = (1 - \beta_j)\bar{P}_{\cdot} + D_j .$$

In Eq. 1, neither Y_{hj} nor P_{hj} are observed. The observed values y_{hj} are related to Y_{hj} by

$$(5) \qquad y_{hj} = Y_{hj} + \upsilon_{hj} N_h$$

where υ_{hj}^2 represents the variance of y_{hj} about Y_{hj}. The sampling variance terms (υ_{hj}^2) were calculated by dividing the variance of the distribution of lengths-at-age j in each sample set $h = 1, \ldots, m$ by the sample sizes (r_{hi}).

The observed proportions male, p_h, are related to the true proportions, P_{hj}, by

$$(6) \qquad p_h = B_h$$

where B_h is a binomial deviate obtained from a sample of n_h individuals drawn from an aggregation of hake with a proportion male of P_{hj}.

The independent variance terms arising through sampling error (υ_{hj}^2) and model error (λ_j^2) are additive, thereby defining

$$(7) \qquad \sigma_{hj}^2 = \lambda_j^2 + \upsilon_{hj}^2 .$$

This completes the definition of our binomial–normal functional model and leads to the likelihood function (Edwards 1972)

$$(8) \qquad L = \prod_{h=1}^{m} \frac{1}{\sqrt{2\pi}\sigma_{hj}} e^{\frac{-(\beta_j P_{hj} + (1 - \beta_j)\bar{P}_{\cdot} + D_j - y_{hj})^2}{2\sigma_{hj}^2}} \times \binom{n_h}{s_h} P_{hj}^{s_h} (1 - P_{hj})^{(n_h - s_h)}$$

without the multiplicative constant

$$(9) \qquad \prod_{h=1}^{m} \frac{n_h!}{s_h!(n_h - s_h)!} .$$

Normally one would proceed by minimizing the negative log-likelihood (Edwards 1972)

$$(10) \qquad l(\Theta) = \sum_{h=1}^{m} \ln(\sqrt{2\pi}\sigma_{hj}) + \frac{(\beta_j P_{hj} + (1 - \beta_j)\bar{P}_{\cdot} + D_j - y_{hj})^2}{2\sigma_{hj}^2} - s_h \ln(P_{hj}) - (n_h - s_h)\ln(1 - P_{hj})$$

with respect to the vector of parameters $\Theta = (\bar{Y}_{\cdot}, \bar{P}_{\cdot j}, \lambda_j^2, P_{1j}, \ldots, P_{mj})$, at which point $l(\Theta) = l(\Theta)_{\min}$ and the maximum likelihood estimates for Θ are obtained. However, Eq. 10 can be an ill-conditioned likelihood function because there exists a set of parameters that permits $l(\Theta) \to -\infty$ as $\sigma_{hj}^2 \to 0$ when all υ_{hj}^2's are ignored, i.e. equal 0. This is similar to the problem that plagues solution of the standard bivariate model with errors in both variables without knowledge of the variance ratio (Schnute 1987).

Nevertheless, when the variance ratio is unknown the standard bivariate model with errors in both variables can be solved for a saddle point that corresponds to a geometric mean regression (Schnute 1987). Analogously, Eq. 10 can be solved for a local minimum which has desirable statistical properties. The solution to Eq. 10 that we propose yields a functional relationship that will range between the limits of an ordinary linear regression model (if no sampling error) and a simple binomial error model (if all error can be explained by binomial sampling error). Intermediate solutions respect the relative degree of normal and binomial error about the Y_{hj}'s and P_{hj}'s, respectively. This behavior stems from the variance of the binomial proportions (P_{hj}'s) being analytically defined by the estimates of P_{hj} and the sample size (n_h).

The first step in finding the local maximum likelihood solution is to provide an analytical expression for the local maximum likelihood estimates of many P_{hj}, $h = 1, \ldots, m$ in terms of the remaining parameters. Appendix B describes an algorithm for obtaining the particular root of the following equation cubic in P_{hj}:

$$(11) \qquad 0 = -P_{hj}^3 - \left\{\left(\frac{\bar{Y}_{\cdot j} - y_{hj}}{\beta_j}\right) - \bar{Y}_{\cdot j} + D_j - 1\right\} P_{hj}^2 + \left\{\left(\frac{\bar{Y}_{\cdot j} - y_{hj}}{\beta_j}\right) - \bar{Y}_{\cdot j} + D_j + \left(\frac{\sigma_{hj}^2 n_h}{\beta_j^2}\right)\right\} P_{hj} - \left\{\frac{\sigma_{hj}^2 s_h}{\beta_j^2}\right\}$$

which, as explained in Appendix A, corresponds to a local maximum likelihood estimate of P_{hj}. This estimator of the P_{hj}'s can be shown to have the property that all estimates are analytically constrained to fall within the domain 0 to 1 as would be desired for a binomial proportion. It then becomes a relatively simple matter to estimate the remaining few parameters numerically.

It is useful to know that if the model data set is simplified by ignoring the individual sampling error variances υ_{hj}^2 such that σ_{hj}^2 is replaced by λ_j^2, then repeating the exercise in Appendix A will show that the expected mean, $\bar{Y}_{\cdot j}$, can be replaced by the sample mean, $\bar{y}_{\cdot j}$, in Eq. 11, i.e., $\bar{y}_{\cdot j}$ will be the expected value of $\bar{Y}_{\cdot j}$ ($\hat{\bar{Y}}_{\cdot j} = \bar{y}_{\cdot j}$). When σ_{hj}^2 is replaced by

TABLE 2. First and second partial derivatives of the likelihood function, $l(\Theta)$, with respect to the numerically estimated parameters $\bar{Y}_{\cdot j}$, β_j, and λ_j^2. If the sampling error variances (υ_{hj}^2) are ignored, then Eq. T7 equates to 0, indicating that $\bar{Y}_{\cdot j}$ and β_j are orthogonal in that case. Equations T4–T9 define the elements of the Hessian matrix which when inverted yields the covariance matrix (Kendall and Stuart 1979). These equations can be useful in efficient derivative-based minimization routines such as Marquardt's algorithm (Press et al. 1986).

$$(T1)\quad \frac{\partial l(\Theta)}{\partial \bar{Y}_{\cdot j}} = \sum_{h=1}^{m} \frac{(\beta_j P_{hj} + (1-\beta_j)\bar{P}_{\cdot} + D_j - y_{hj})}{\sigma_{hj}^2}$$

$$(T2)\quad \frac{\partial l(\Theta)}{\partial \beta_j} = \sum_{h=1}^{m} \frac{(\beta_j P_{hj} + (1-\beta_j)\bar{P}_{\cdot} + D_j - y_{hj})(P_{hj} - \bar{P}_{\cdot})}{\sigma_{hj}^2}$$

$$(T3)\quad \frac{\partial l(\Theta)}{\partial \lambda_j^2} = \sum_{h=1}^{m} \frac{1}{2\sigma_{hj}^2} - \frac{(\beta_j P_{hj} + (1-\beta_j)\bar{P}_{\cdot} + D_j - y_{hj})^2}{2\sigma_{hj}^4}$$

$$(T4)\quad \frac{\partial^2 l(\Theta)}{\partial^2 \bar{Y}_{\cdot j}} = \sum_{h=1}^{m} \frac{1}{\sigma_{hj}^2}$$

$$(T5)\quad \frac{\partial^2 l(\Theta)}{\partial^2 \beta_j} = \sum_{h=1}^{m} \frac{(P_{hj} - \bar{P}_{\cdot})^2}{\sigma_{hj}^2}$$

$$(T6)\quad \frac{\partial^2 l(\Theta)}{\partial^2 \lambda_j^2} = -\sum_{h=1}^{m} \frac{1}{2\sigma_{hj}^4} - \frac{(\beta_j P_{hj} + (1-\beta_j)\bar{P}_{\cdot} + D_j - y_{hj})^2}{\sigma_{hj}^6}$$

$$(T7)\quad \frac{\partial^2 l(\Theta)}{\partial \bar{Y}_{\cdot j}\, \partial \beta_j} = \sum_{h=1}^{m} \frac{(P_{hj} - \bar{P}_{\cdot})}{\sigma_{hj}^2}$$

$$(T8)\quad \frac{\partial^2 l(\Theta)}{\partial \bar{Y}_{\cdot j}\, \partial \lambda_j^2} = -\sum_{h=1}^{m} \frac{(\beta_j P_{hj} + (1-\beta_j)\bar{P}_{\cdot} + D_j - y_{hj})}{\sigma_{hj}^4}$$

$$(T9)\quad \frac{\partial^2 l(\Theta)}{\partial \beta_j\, \partial \lambda_j^2} = -\sum_{h=1}^{m} \frac{(\beta_j P_{hj} + (1-\beta_j)\bar{P}_{\cdot} + D_j - y_{hj})(P_{hj} - \bar{P}_{\cdot})}{\sigma_{hj}^4}$$

λ_j^2 the estimates for the main parameters of interest are asymptotically unbiased. That is, the binomial–normal functional model tends toward an ordinary regression model as the n_h's $\rightarrow \infty$ (Appendix C).

Readers should also realize that our choice to let P_{hj} represent the proportion male, rather than the proportion female, has no bearing on the statistical conclusions of the binomial–normal functional model or the value of $l(\Theta)_{\min}$. The only effect is to change the local maximum likelihood estimates of $\bar{P}_{\cdot}$ and the P_{hj}'s to $1 - \bar{P}_{\cdot}$ and $1 - P_{hj}$, respectively; and the values of β_j and β_{ij} to $-\beta_j$ and $-\beta_{ij}$, respectively.

Our model is parametrically nonlinear; therefore, we used the direct-search SIMPLEX algorithm of Nelder and Mead (1965) as implemented by Mittertreiner and Schnute (1985) to minimize the likelihood function $l(\Theta)$ for our contrived data (Table 3; Fig. 3), as well as the likelihood functions $l(\Theta_1)$ and $l(\Theta_2)$ in our formal analyses. We then inverted the matrix of second partial derivatives of the likelihood function $l(\Theta)$ with respect to the parameters (Hessian matrix) to generate the asymptotic covariance matrix (Kendall and Stuart 1979). The elements of the covariance matrix indicated multinormal behavior (Kendall and Stuart 1979) of the likelihood function near $l(\Theta)_{\min}$ in all analyses; thus we report standard errors for all the numerically estimated parameters of Θ_1 and Θ_2. Similar behavior of the covariance matrix for ordinary data sets is expected but not guaranteed.

The first and second partial derivatives of the likelihood function with respect to the numerically estimated parameters (Table 2) will be useful to those who have a derivative-based minimization algorithm available to them. These derivatives treat the P_{hj}'s as constants, i.e. they are not parametrically linked to $\bar{Y}_{\cdot j}$, β_j, and λ_j^2 as our model describes. Consequently, a covariance matrix determined from a Hessian matrix built with Eq. T4–T9 will be conditional on the values for the P_{hj}'s being true. Confidence limits for $\bar{Y}_{\cdot j}$, β_j, and λ_j^2 obtained using Eq. T4–T9 will thus be narrower (i.e. more optimistic) than those obtained using a numerically calculated Hessian (e.g. Mittertreiner and Schnute 1985) with the P_{hj}'s parametrically linked to $\bar{Y}_{\cdot j}$, β_j, and λ_j^2.

Mean Lengths-at-Age versus Sex or Age Composition

Our data on mean lengths-at-age (y_{hj}, $j = 3, \ldots, 14^{>}$), proportions-at-age (p_{hi}, $i = 3, \ldots, 14^{>}$), and proportion male (p_h) obtained from the $m = 29$ sample sets would allow a total of 156 individual analyses of the relationship between mean lengths-at-age and the proportion male or proportions-at-age for both males and females. Rather than proceed with such a large number of individual analyses, we chose to proceed with four analyses (two each for males and females) where the main parameters of interest ($\bar{Y}_{\cdot j}$, λ_j^2, β_j, or β_{ij}) were modelled as functions of age. Structuring our analyses in this fashion increased model parsimony, effectively increased overall sample size, and generated results which showed the main trends in the relationships. In the analyses which follow, only data records where an observed mean length-at-age (y_{hj}) is based on a sample size (r_{hi}) of two or more individuals, such that υ_{hj} is defined, are used.

Firstly, for both males and females, we analyzed the 12 relationships between mean lengths-at-ages (Y_{hj}, $j = 3, \ldots, 14^{>}$) and the proportion male (P_{hj}) using data obtained from each sample set $h = 1, \ldots, m$. We required the values of $\bar{Y}_{\cdot j}$, λ_j^2, and β_j to lie on reparameterized von Bertalanffy curves (Schnute and Fournier 1980; Schnute 1981) according to

$$(12)\quad \bar{Y}_{\cdot j} = \bar{Y}_{\cdot 3} + (\bar{Y}_{\cdot 14} - \bar{Y}_{\cdot 3})\frac{(1 - K^{j-3})}{(1 - K^{14-3})},$$

$$(13)\quad \lambda_j^2 = \lambda_3^2 + (\lambda_{14}^2 - \lambda_3^2)\frac{(1 - K^{j-3})}{(1 - K^{14-3})},$$

and

$$(14)\quad \beta_j = \beta_3 + (\beta_{14} - \beta_3)\frac{(1 - K^{j-3})}{(1 - K^{14-3})},$$

respectively, where K represents the annual rate of change in the value of each of $\bar{Y}_{\cdot j}$, λ_j^2, and β_j. Approximate local maximum likelihood estimates for the parameter vector $\Theta_1 = (\bar{Y}_{\cdot 3}, \bar{Y}_{\cdot 14}, \lambda_3^2, \lambda_{14}^2, \beta_3, \beta_{14}, K, \bar{P}_{\cdot}, P_{1j}, \ldots, P_{mj})$ were obtained by minimizing

$$(15)\quad l(\Theta_1) = \sum_{h=1}^{m}\sum_{j=3}^{14} \ln(\sqrt{2\pi}\,\sigma_{hj}) + \frac{(\beta_j P_{hj} + (1-\beta_j)\bar{P}_{\cdot} + D_j - y_{hj})^2}{2\sigma_{hj}^2} - s_h \ln(P_{hj}) - (n_h - s_h)\ln(1 - P_{hj}),$$

noting, however, that missing data records (i.e. when $r_{hi} < 2$) result in the likelihood function $l(\Theta_1)$ being evaluated from fewer than the maximum 29×12 terms.

TABLE 3. Parameter estimates for the binomial–normal functional model portrayed in Fig. 3. For clarity, the $m = 26$ contrived "observed" data records in the Fig. 3 example are listed in ascending order of the values of p_h. For comparison, the ordinary regression model yielded $\hat{P}_{hj} = p_h$, $\hat{\bar{P}}_{\cdot} = \bar{p}_{\cdot}$, $\hat{\bar{Y}}_{\cdot j} = \bar{y}_{\cdot j}$, $\hat{\beta}_j = -4.78$ cm, and $\hat{\lambda}_j^2 = 3.57$ cm^2. The model with binomial sampling error only yielded $\hat{\beta}_j = -18.35$ cm and $\hat{\lambda}_j^2 = 0$ cm^2. The binomial–normal functional model yielded $\hat{\beta}_j = -8.69$ cm and $\hat{\lambda}_j^2 = 1.74$ cm^2. See Table 1 for key to column headings.

	Observed					Expected	
Set (h)	n_h	s_h	p_h	y_{hj}	v_{hj}^2	$\hat{P}_{hj}$	$\hat{Y}_{hj}$
1	51	8	0.157	51.00	0.125	0.164	51.55
2	27	5	0.185	49.00	0.400	0.237	50.91
3	21	5	0.238	52.53	0.003	0.193	51.29
4	167	40	0.240	49.93	0.003	0.245	50.85
5	29	8	0.276	52.33	0.003	0.233	50.95
6	18	5	0.278	52.00	3.600	0.256	50.74
7	170	51	0.300	50.32	0.001	0.300	50.36
8	16	5	0.313	51.00	0.137	0.284	50.51
9	24	8	0.333	52.33	0.042	0.267	50.66
10	556	197	0.354	47.55	0.002	0.359	49.85
11	21	8	0.381	47.87	0.107	0.448	49.08
12	97	37	0.381	50.76	0.012	0.370	49.76
13	13	5	0.385	47.50	0.057	0.494	48.68
14	13	5	0.385	49.50	0.100	0.391	49.57
15	13	5	0.385	49.50	0.100	0.391	49.57
16	40	18	0.450	50.21	0.308	0.426	49.28
17	558	272	0.488	46.80	0.002	0.492	48.70
18	639	356	0.557	46.25	0.001	0.561	48.10
19	13	8	0.615	47.00	0.001	0.647	47.35
20	8	5	0.625	45.50	0.057	0.742	46.52
21	16	10	0.625	48.25	0.040	0.593	47.82
22	178	126	0.708	45.83	0.006	0.713	46.78
23	29	21	0.724	49.40	0.046	0.646	47.36
24	21	16	0.762	50.83	0.435	0.617	47.61
25	13	10	0.769	46.75	0.086	0.749	46.46
26	5	5	1.000	49.50	0.100	0.602	47.74
			$\bar{p}_{\cdot}$	$\bar{y}_{\cdot j}$		$\hat{\bar{P}}_{\cdot}$	$\hat{\bar{Y}}_{\cdot j}$
			0.458	49.21		0.439	49.16

Minimizing Eq. 15 provides only an approximate estimate of Θ_1 because Eq. 15 ignores possible covariance of the repeated estimates of P_{hj} for single observed estimates of p_h (s_h/n_h) with age j. This covariance results from Eq. 12 to 14 redefining the binomial–normal functional model such that Eq. 11 is no longer an exact estimator of the P_{hj}'s. We assume any such covariance to be negligible.

Secondly, for both males and females, we analyzed the 144 relationships between the 12 mean lengths-at-ages $j = 3, \ldots 14^{>}$ (Y_{hij}) and each of the 12 proportion-at-ages $i = 3, \ldots, 14^{>}$ (P_{hij}) using data obtained from each sample set $h = 1, \ldots, m$. In this analysis we use the subscript i to index the age-classes $i = 3, \ldots, 14^{>}$ whose proportions influence mean length-at-age and the subscript $j = 3, \ldots, 14^{>}$ to index the mean length-at-age influenced. This modification requires a modest revision of the subscripts in our binomial–normal functional model such that

$$Y_{hij} = \beta_{ij} P_{hij} + (1 - \beta_{ij}) \bar{P}_{\cdot i} + D_{ij} \quad (16)$$

with

$$D_{ij} = \bar{Y}_{\cdot j} - \bar{P}_{\cdot i}\,, \quad (17)$$

$$y_{hj} = Y_{hij} + \sigma_{hj} N_h\,, \quad (18)$$

and

$$p_{hi} = B_h \quad (19)$$

where B_h is a binomial deviate obtained from a sample of n_h individuals drawn from an aggregation of hake with proportions-at-age P_{hij}.

As for the previous analysis, we required the values of $\bar{Y}_{\cdot j}$ and λ_j^2 to lie on von Bertalanffy curves (Schnute and Fournier 1980; Schnute 1981) according to Eq. 12 and 13 in order to obtain model parsimony. We similarly required the values of β_{ij} to lie on von Bertalanffy curves across the two dimensions i and j as

$$\beta_{ij} = \beta_{i,3} + (\beta_{i,14} - \beta_{i,3}) \frac{(1 - K^{j-3})}{(1 - K^{14-3})} \quad (20)$$

where

$$\beta_{i,3} = \beta_{3,3} + (\beta_{14,3} - \beta_{3,3}) \frac{(1 - K^{i-3})}{(1 - K^{14-3})} \quad (21)$$

and

$$\beta_{i,14} = \beta_{3,14} + (\beta_{14,14} - \beta_{3,14}) \frac{(1 - K^{i-3})}{(1 - K^{14-3})} \quad (22)$$

Values for the mean proportions-at-age ($\bar{P}_{\cdot}$, $i = 3, \ldots, 14^{>}$) could not be linked by a simple parameterization because year-

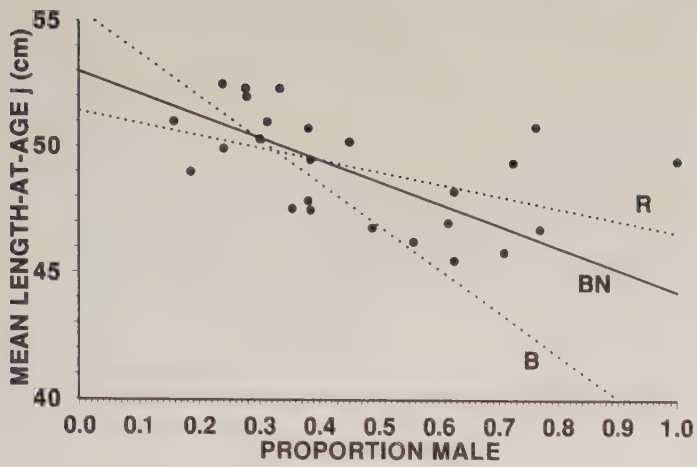


FIG. 3. Comparison of the linear relationship between the proportion of males in a sample set and mean length-at-age j as estimated by an ordinary linear regression model (R), a model with binomial sampling error (B), and a binomial–normal functional model (BN). The ordinary regression model fails to account for uncertainty in the estimates of p_h which is high for small sample sizes (n_h). Conversely, the model with only binomial sampling error implies that mean length-at-age j is known exactly. The contrived data used in this example plot are given in Table 3.

class strength is a somewhat random phenomenon for offshore Pacific hake (Smith et al. 1990). Approximate local maximum likelihood estimates for the parameter vector $\Theta_2 = (\bar{Y}_{\cdot 3}, \bar{Y}_{\cdot 14}, \lambda_3^2, \lambda_{14}^2, \beta_{3,3}, \beta_{3,14}, \beta_{14,3}, \beta_{14,14}, K, \bar{P}_{\cdot 3}, \ldots, \bar{P}_{\cdot 14}, P_{1ij}, \ldots, P_{mij})$ were obtained by minimizing

$$(23)\quad l(\Theta_2) = \sum_{h=1}^{m}\sum_{i=3}^{14}\sum_{j=3}^{14} \ln(\sqrt{2\pi}\,\sigma_{hj}) + \frac{(\beta_{ij}P_{hij} + (1-\beta_{ij})\bar{P}_{\cdot i} + D_{ij} - y_{hj})^2}{2\sigma_{hj}^2} - r_{hi}\ln(P_{hij}) - (n_h - r_{hi})\ln(1 - P_{hij})$$

where the values for each P_{hij} are obtained as the appropriate root of

$$(24)\quad 0 = -P_{hij}^3 - \left\{\left(\frac{\bar{Y}_{\cdot j} - y_{hj}}{\beta_{ij}}\right) - \bar{Y}_{\cdot j} + D_{ij} - 1\right\}P_{hij}^2 + \left\{\left(\frac{\bar{Y}_{\cdot j} - y_{hj}}{\beta_{ij}}\right) - \bar{Y}_{\cdot j} + D_{ij} + \left(\frac{\sigma_{hj}^2 n_h}{\beta_{ij}^2}\right)\right\}P_{hij} - \left\{\frac{\sigma_{hj}^2 r_{hi}}{\beta_{ij}^2}\right\}$$

as described in Appendix B if P_{hij}, β_{ij}, and D_{ij} are substituted for P_{hj}, β_j, and D_j, respectively. Missing data records (i.e. when $r_{hi} < 2$) result in the likelihood function $l(\Theta_2)$ being evaluated from fewer than the maximum $29 \times 12 \times 12$ terms.

A strict analysis of our age composition data according to the model described here should acknowledge that P_{hij} is no longer a binomial variate, but is instead a multinomial variate, by imposing the constraint

$$(25)\quad \sum_{i=3}^{14} P_{\cdot i \cdot} = 1$$

over all 29×12 combinations of h and j. Unfortunately, as described, the model structure is not flexible enough to permit exact adherence to this constraint if we are to use Eq. 24 to generate analytical estimates of the P_{hij}'s. Solving for the nearly $29 \times 12 \times 12$ P_{hij}'s numerically is not practical, so we relax the constraint of Eq. 25 and impose a less rigorous constraint of

$$(26)\quad \sum_{i=3}^{14} \bar{P}_{\cdot i} = 1$$

in order to keep the model solvable. We then establish a posteriori that the sums of Eq. 25 all fall between 0.98 and 1.02, thereby justifying our modified constraint. Similarly to the argument regarding the minimization of $l(\Theta_1)$, minimizing $l(\Theta_2)$ provides only approximate estimates of Θ_2 because Eq. 23 ignores possible covariance of the repeated estimates of P_{hij} with the age influenced (j), and the repeated estimates of Y_{hij} with the age of influence (i), for single observed estimates of p_{hi} and y_{hj}, respectively. Again we assume any such covariance to be negligible.

Results

We introduce our binomial–normal functional model by first applying it to a set of $m = 26$ contrived data records ($h = 1, \ldots, m$) (Table 3). We chose to present this example with a mixture of small and large binomial sample sizes (n_h) to emphasize the importance of considering binomial sampling error when analyzing such data for a simple linear relationship. Comparison of the binomial–normal functional model with the ordinary regression model (Fig. 3) demonstrates that ignoring binomial error can lead to misinterpretation of the data in Table 3. As the sample sizes (n_h) from which the binomial proportions p_h are determined increase, the binomial–normal functional model approaches an ordinary regression model. Conversely, as the sample sizes (n_h) from which the binomial proportions p_h are determined decrease, the binomial–normal functional model loses ability to estimate the variance (λ_j^2) associated with the estimates of mean length-at-age j.

For the example given in Table 3 and Fig. 3 the values for $l(\Theta)$ were 1833.67 (not a minimum) for ordinary regression but with the υ_{hj}^2's > 0 (R), 1832.79 (a local minimum) for the binomial–normal functional model (BN), and 1828.15 (a global minimum) for a model with binomial sampling error and $\lambda_j^2 = 0.0$ cm^2 (B). Statistical inference based on likelihood ratios (see table 1 of Schnute and Groot 1992) suggests that the "best" model for these data is the one with the smallest $l(\Theta)_{\min}$, i.e. model B. Consistent with this conclusion, the confidence region (Schnute and Groot 1992) for $\lambda_j^2 = 3.57$ cm^2 obtained with the BN model embraces $\lambda_j^2 = 0.0$ cm^2.

There is no simple rule for assessing the need to use the binomial–normal functional model instead of ordinary regression. However, using other trial data sets with $m = 26$, we found that if all binomial proportions were estimated from sample sizes of about 50 or more, then an ordinary regression model could adequately replace the binomial–normal functional model. We recommend that if binomial proportions are in general based on quite small sample sizes, then use of the binomial–normal functional model should be considered. As few as one outlier proportion based on a small sample size can result in the binomial–normal functional model giving a fairer result than that given by an ordinary regression model. It is important to understand that increasing the number of samples (h) is not a substitute for binomial proportions being estimated from small sample sizes (n_h).

Preliminary analyses of the relationships between mean

TABLE 4. Local maximum likelihood estimates and approximate standard errors (SE) for the numerically estimated parameters of a binomial–normal functional model which demonstrates the relationship between the sex or age compositions of sample sets of hake and mean lengths-at-age for those sample sets. The analytically estimated sets of nearly 29×12 P_{hj}'s and nearly $29 \times 12 \times 12$ P_{hij}'s have no interpretation value and are not reported. See Table 1 for key to parameters.

	Male		Female	
	Estimate	SE	Estimate	SE
Sex composition model				
$\bar{Y}_{\cdot 3}$	43.71	0.46	43.26	0.54
$\bar{Y}_{\cdot 14}$	52.58	0.33	56.26	0.36
K	0.850	0.03	0.837	0.02
β_3	−7.09	1.44	−8.50	1.74
β_{14}	−7.09	1.44	−8.50	1.74
λ_3^2	2.71	0.31	4.49	0.46
λ_{14}^2	2.71	0.31	4.49	0.46
$\bar{P}_{\cdot}$	0.407	0.11	0.387	0.11
Age composition model				
$\bar{Y}_{\cdot 3}$	43.23	0.13	42.68	0.13
$\bar{Y}_{\cdot 14}$	52.52	0.11	56.31	0.12
K	0.840	0.01	0.838	0.01
$\beta_{3,3}$	−1.21	1.70	−1.48	1.83
$\beta_{14,3}$	1.43	1.66	1.64	1.84
$\beta_{3,14}$	−2.81	1.62	−4.29	1.79
$\beta_{14,14}$	2.23	1.36	3.45	1.56
λ_3^2	2.37	0.22	2.96	0.26
λ_{14}^2	3.63	0.18	6.33	0.26
$\bar{P}_{\cdot 3}$	0.001	0.13	0.001	0.14
$\bar{P}_{\cdot 4}$	0.079	0.13	0.116	0.14
$\bar{P}_{\cdot 5}$	0.104	0.13	0.214	0.14
$\bar{P}_{\cdot 6}$	0.188	0.13	0.131	0.14
$\bar{P}_{\cdot 7}$	0.228	0.13	0.155	0.14
$\bar{P}_{\cdot 8}$	0.124	0.13	0.146	0.14
$\bar{P}_{\cdot 9}$	0.087	0.13	0.079	0.14
$\bar{P}_{\cdot 10}$	0.053	0.13	0.052	0.14
$\bar{P}_{\cdot 11}$	0.040	0.13	0.019	0.14
$\bar{P}_{\cdot 12}$	0.048	0.13	0.065	0.14
$\bar{P}_{\cdot 13}$	0.022	0.13	0.018	0.14
$\bar{P}_{\cdot 14}$	0.026	0.13	0.004	0.14

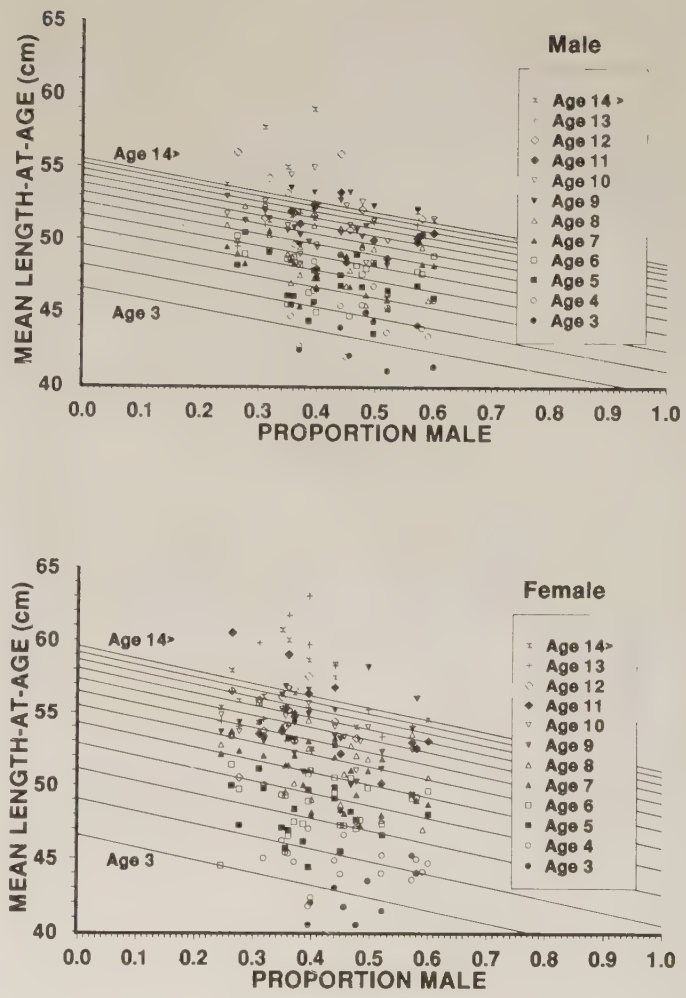


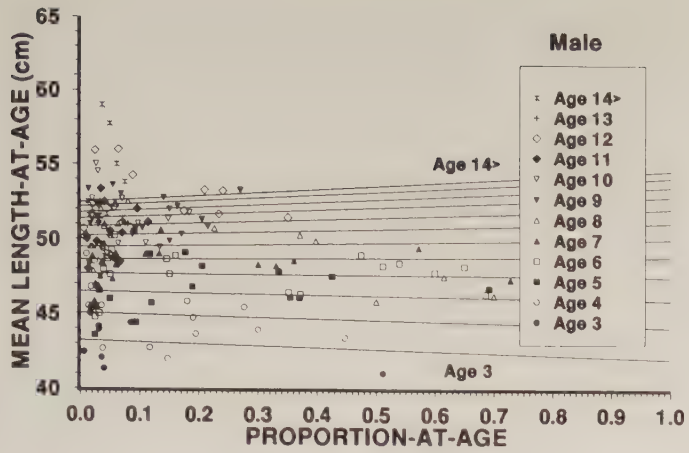
FIG. 4. Likelihood-based linear relationships between the proportion of males within sample sets and the mean lengths-at-ages 3 to $14^>$ within those sample sets. To obtain model parsimony, all values of β_j, $j = 3, \ldots, 14^>$ (i.e. the slopes), for these 12 relationships were constrained to be equal.

lengths-at-age and sex composition indicated trends of increasingly negative slopes (β_j's) with increasing age. However, in the analyses we report (Fig. 4; Table 4), we constrained the β_j's and λ_j^2's to be equal for all age-classes to obtain model parsimony. The significant trends of decreasing mean lengths-at-ages $j = 3, \ldots, 14^>$ (β_j's, see Table 4) with an increase in the proportion of males in a sample set for both males and females (Fig. 4) provide strong evidence that offshore Pacific hake aggregate according to size. With male hake being smaller than female hake, our interpretation is that as the proportion of males in an aggregation increases, mean lengths for all ages tend to decrease due to the aggregations being increasingly structured according to the size of males.

The analyses of the relationships between mean lengths-at-age and age composition (Fig. 5) produced results that were not statistically conclusive (Table 4), yet demonstrated trends that are consistent with the concept that Pacific hake aggregate by size. For example, as the proportion of 3-yr-old (age of influence) hake in a sample set increases, the mean length-at-age 3 (age influenced) decreases. Conversely, as the proportion of $14^>$-yr-old (age of influence) hake in a sample set increases, the mean length-at-age $14^>$ (age influenced) increases. These features are apparent in Fig. 5 as the negative slope for age 3 ($\beta_{3,3}$), the positive slope for age $14^>$ ($\beta_{14,14}$), and the gradual trend toward increasing slopes from ages 3 to $14^>$.

The gradual change in slope with increasing age in Fig.5 displays the pattern of β_{ij}'s only for the subset of values when $i = j$. The values for the full grid of β_{ij}'s (Fig. 6) support the interpretation that as the number of younger (smaller) fish in an aggregation increases, the tendency is a decrease in the mean length-at-age for all ages in that aggregation. Conversely, as the number of older (larger) fish in an aggregation increases, the tendency is an increase in the mean length-at-age for all ages in that aggregation. Although the values for the β_{ij}'s have broad confidence limits (Table 4) which include $\beta_{ij} = 0$, these results provide credible evidence in support of the concept that aggregations of fish organize by size. We believe that the weakness of the statistical conclusions of the "age composition" analysis results from most of the estimated proportions-at-age (p_{hi}) being based on small binomial sample sizes (r_{hi}). This results in large estimates of binomial sampling variance (ϕ_{hij}^2) which in turn weakens the power of the statistical analyses to reject the null hypothesis that $\beta_{ij} = 0$ (see Peterman 1990).

The effect of the tendency for hake to form aggregations based on size is to add variance to estimates of mean lengths-at-age from different sample sets collected from the same



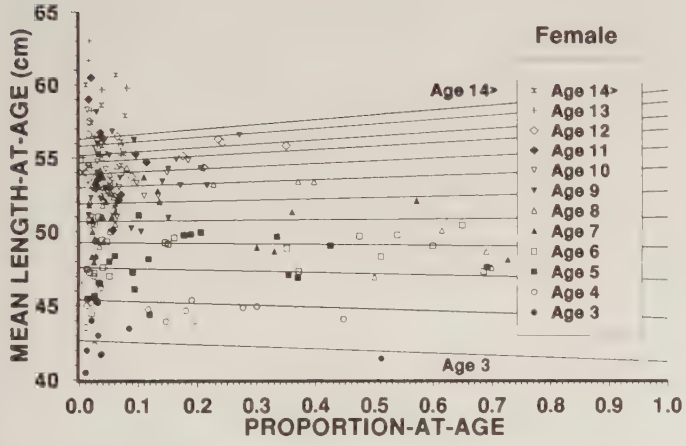
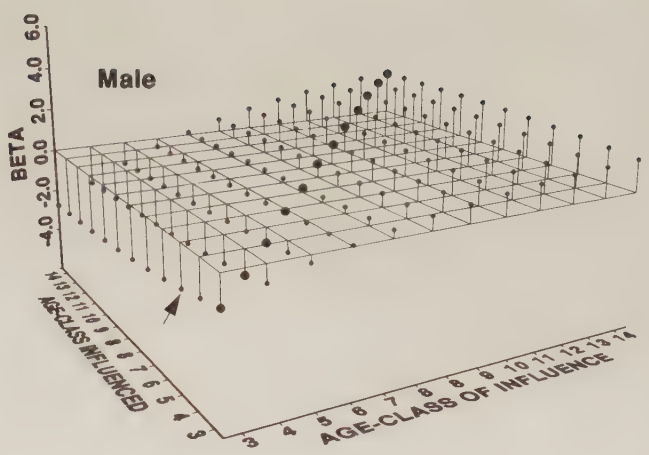


FIG. 5. Likelihood-based linear relationships between the proportions-at-ages 3 to $14^{>}$ within sample sets and the mean lengths-at-age for those same ages 3 to $14^{>}$ (i.e. $i = j$) within those sample sets. The values of β_{ij} (i.e. the slopes) for these 12 relationships correspond to those values of β_{ij} indicated by the enlarged solid circles along the $i = j$ diagonal of Fig. 6.



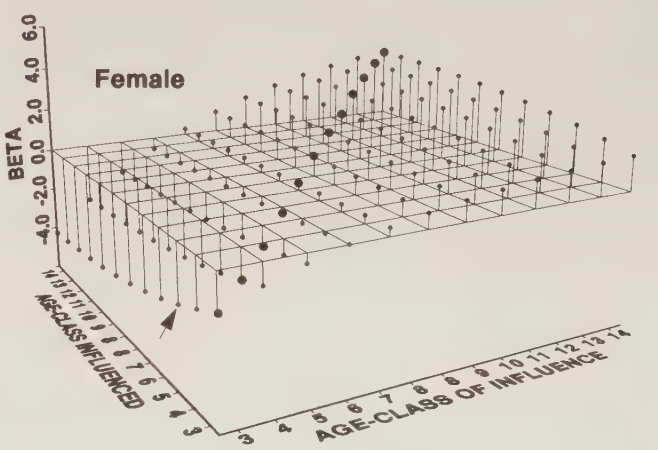


FIG. 6. Grid of 144 values for β_{ij} (BETA) for all combinations of i, $j = 3, \ldots, 14^{>}$. These plots can be interpreted with the following explanation using the value $\beta_{3,5}$ indicated by the arrow as an example. As the proportion of the age-class of influence (age 3) increases within a sample set, the mean length of the age-class influenced (age 5) tends to be modified at the rate of $\beta_{3,5}$ cm (-1.75 and -2.45 cm for males and females, respectively) over the domain (0 to 1) of porportions-at-age 3 (i.e., the age-class of influence). Note that, in general, increased proportions of younger (smaller) hake in a sample set tend to decrease mean lengths-at-age whereas increased proportions of older (larger) hake in a sample set tend to increase mean lengths-at-age. Those values of β_{ij} indicated by the enlarged solid circles along the $i = j$ diagonal correspond to the 12 linear relationships portrayed in Fig. 5.

general population. However, the data in Fig. 4 and 5 indicate that the tendency to organize by size accounts for only a small component of the total variance among estimates of mean lengths-at-age for the same general population. After the variance due to the sex or age composition of sample sets is removed, the remaining variance in mean length-at-age estimates from sample sets remains high (Fig. 7). The lesson from the analyses presented here is that sampling programs designed to collect reliable sex–age–size data from a population of interest should recognize aggregations as the basic sampling unit. If feasible, a moderately larger number of sample sets should be collected because confidence in mean length-at-age estimates increases in proportion to $\sqrt{m}$. Sampling programs should also be organized to sample aggregations representing a large diversity of sex or age compositions.

Discussion

Sampling problems arising from hake aggregation organizing by size first came to our attention in an analysis of trends in offshore hake mean lengths-at-age over time (Smith et al. 1990). In that analysis we were unable to normalize the residual variance of our model and speculated that such a sampling artifact might exist. We specifically suggested that dominant age-classes (and consequently, dominant length-classes) could be the focus of a fishery (or research survey) such that the length composition of adjacent age-classes within the catch could be biased toward that of the dominant age-class. The results of this study are consistent with that speculation. However, we wish to revise the previous suggestion to clarify that since fishermen and scientists do not know the age of hake they are targeting, it must be that length-classes are the focus of the fishery, not age-classes.

As mentioned earlier, the concept that fish organize into aggregations or schools based on size is not new. However, we are not aware of any statistical studies of this phenomenon with respect to sex–age–size relationships among fish within aggregations. Observations similar to ours have been reported by Freon (1984) who elaborated on the dynamics of such organi-

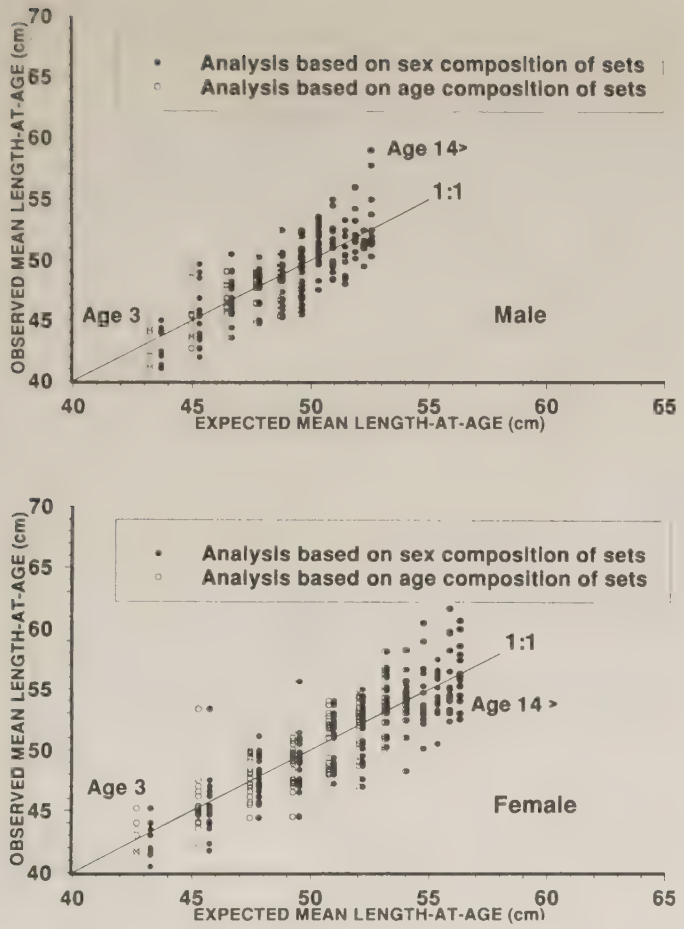


FIG. 7. Variation in observed mean lengths-at-ages 3 to $14^{>}$ obtained from individual sample sets (y_{hj}, circles) expressed relative to the expected mean lengths-at-ages 3 to $14^{>}$ ($\bar{Y}_{\cdot j}$, 1:1 line) estimated by the analyses based on the sex and age compositions of the sample sets. The average standard deviations of the y_{hj}'s about $\bar{Y}_{\cdot j}$ were 1.9 and 2.4 cm for males and females, respectively.

zational behavior in relation to the growth and reproduction of some tropical pelagic species. Freon (1984, 1985) further explained how such organizational behavior has the potential to cause problems in the estimation of sex–age–size relationships for a population of fish using data collected from fish organized into schools. The thrust of the contribution of Freon (1984, 1985) is that if fish schools are oganized by size, then trajectories of growth and growth variance obtained from samples collected from only a few schools will not accurately reflect age-class mean size or variance in size. These conclusions are entirely consistent with our results.

Our contribution differs from that of Freon (1984, 1985) in that we have presented formal statistical analyses of the relationship between the sex and age composition of hake aggregation and estimates of mean lengths-at-age from those aggregations. Consistent with the conceptual arguments of Freon (1984, 1985) regarding the age-class and size composition of schools, our analyses provide statistical evidence that aggregations of Pacific hake are indeed likely to be somehow organized by size. Consequently, sampling for sex–age–size relationships in hake should focus on aggregations of fish, not individual fish, as the basic sampling unit. Such a two-stage sampling program for wild populations is generally the preferred strategy for many reasons other than the organizational structure of fish aggregations (Pielou 1974). Habitat diversity, stock diversity, migration patterns, predator and prey distribution, and reproductive behavior generally result in a nonrandom distribution of individuals of many fish species, including Pacific hake.

From a statistical perspective, we anticipate that the binomial–normal functional model we introduce in this paper can find pertinent use in fisheries and ecological science. Fisheries and ecological data often consist of counts of some species or item from which proportional relationships are constructed. Appropriate statistical analyses of such data might demand that the error in estimation of those proportions from sample counts be acknowledged, particularly if the proportions are based on relatively small counts. The potential cost of ignoring such binomial sampling error, for example by choosing to use ordinary regression analysis, is an incorrect judgment that a relationship between two variates is statistically significant. Because it is the practice of researchers to make interpretive conclusions about natural processes based on such statistical conclusions, such statistical errors must be avoided. It is our obligation as researchers to attempt to prevent erroneous conclusions about natural processes finding their way into scientific literature due to inappropriate statistical analysis.

Acknowledgments

We thank Dr. T. Mulligan for a constructive criticism of an earlier version of this manuscript and Drs. D. Noakes and J. Schnute for fruitful discussions of this model.

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Appendix A: Likelihood Estimates

This appendix explains how the local maximum likelihood estimate for any P_{hj} is derived in terms of estimates for the remaining model parameters $\bar{Y}_{.j}$, $\bar{P}_{.}$, λ_j^2, and β_j. To find the local maximum likelihood estimate for P_{hj}, we must set

$$\text{(A1)} \quad \frac{\partial l(\Theta)}{\partial P_{hj}} = 0$$

and then solve for P_{hj}. The negative log-likelihood function ($l(\Theta)$) in Eq. 10 can be expressed as the sum of likelihood functions for each of the normal and binomial data types:

$$\text{(A2)} \quad l(\Theta) = \tau_1 - \tau_2$$

where

$$\text{(A3)} \quad \tau_1 = \sum_{h=1}^{m} \ln(\sqrt{2\pi}\sigma_{hj}) + \frac{(\beta_j P_{hj} + (1 - \beta_j)\bar{P}_{.} + D_j - y_{hj})^2}{2\sigma_{hj}^2}$$

and

$$\text{(A4)} \quad \tau_2 = \sum_{h=1}^{m} s_h \ln(P_{hj}) + (n_h - s_h)\ln(1 - P_{hj}).$$

Now

$$\text{(A5)} \quad \frac{\partial \tau_1}{\partial P_{hj}} = \frac{1}{\sigma_{hj}^2}\{\beta_j(\beta_j P_{hj} + (1 - \beta_j)\bar{P}_{.} + D_j - y_{hj}) + (1 - \beta_j)(\bar{P}_{.} + D_j - \bar{y}_{.j})\}$$

and

$$\text{(A6)} \quad \frac{\partial \tau_2}{\partial P_{hj}} = \left(\frac{s_h}{P_{hj}}\right) - \left(\frac{n_h - s_h}{1 - P_{hj}}\right)$$

which leads to

$$\text{(A7)} \quad 0 = -P_{hj}^3 - \left\{\left(\frac{\bar{P}_{.} + D_j - y_{hj}}{\beta_j}\right) - \bar{P}_{.} - 1\right\}P_{hj}^2 + \left\{\left(\frac{\bar{P}_{.} + D_j - y_{hj}}{\beta_j}\right) - \bar{P}_{.} + \left(\frac{\sigma_{hj}^2 n_h}{\beta_j^2}\right)\right\}P_{hj} - \left\{\frac{\sigma_{hj}^2 s_h}{\beta_j^2}\right\}$$

when Eq. A1 is expressed as a polynomial cubic in P_{hj}.

A complication with Eq. A7 is that solving for P_{hj} requires an estimate of $\bar{P}_{.}$ which must be obtained as

$$\text{(A8)} \quad \hat{\bar{P}}_{.} = \frac{1}{m}\sum_{h=1}^{m} \hat{P}_{hj}$$

and therefore must be calculated using the estimates for P_{hj} obtained by solving Eq. A7 for the root which corresponds to a local maximum likelihood estimate of P_{hj}. During minimization, updated estimates of $\hat{\bar{P}}_{.}$ can be obtained using Eq. A8.

We can provide an estimate of D_j as follows:

$$\text{(A9)} \quad \frac{\partial l(\Theta)}{\partial P_{hj}} = 0;$$

thus

$$\text{(A10)} \quad \frac{1}{m}\sum_{h=1}^{m} \frac{\partial l(\Theta)}{\partial P_{hj}} = 0.$$

This leads to

$$(A11)\quad 0 = \frac{1}{m}\left\{\sum_{h=1}^{m}\frac{y_{hj}}{\sigma_{hj}^2} - \beta_j\sum_{h=1}^{m}\frac{P_{hj}}{\sigma_{hj}^2} + \sum_{h=1}^{m}\frac{1}{\sigma_{hj}^2} \times (\bar{P}.(\beta_j - 1) - D_j)\right\} - \frac{1}{m}\sum_{h=1}^{m}\left(\frac{s_h}{P_{hj}} - \frac{(n_h - s_h)}{(1-P_{hj})}\right).$$

Since s_h is a binomial random variable with an expected value of $n_h P_{hj}$, i.e.

$$(A12)\quad E[s_h] = n_h P_{hj},$$

then

$$(A13)\quad E\left[\frac{1}{m}\sum_{h=1}^{m}\left(\frac{s_h}{P_{hj}} - \frac{(n_h - s_h)}{(1 - P_{hj})}\right)\right] = 0$$

and we obtain

$$(A14)\quad \hat{D}_j = \frac{\sum_{h=1}^{m}\frac{y_{hj}}{\hat{\sigma}_{hj}^2} - \hat{\beta}_j\sum_{h=1}^{m}\frac{\hat{P}_{hj}}{\hat{\sigma}_{hj}^2}}{\sum_{h=1}^{m}\frac{1}{\hat{\sigma}_{hj}^2}} + \hat{\bar{P}}.(\hat{\beta}_j - 1).$$

Likewise, to find the local maximum likelihood estimates for $\bar{Y}_{.j}$ and β_j in terms of estimates of the remaining parameters, we set the right sides of Eq. T1 and T2 (Table 2) equal to 0 and arrive at

$$(A15)\quad \hat{\bar{Y}}_{.j} = \hat{\beta}_j\hat{\bar{P}}. - \frac{\hat{\beta}_j\sum_{h=1}^{m}\frac{\hat{P}_{hj}}{\hat{\sigma}_{hj}^2} - \sum_{h=1}^{m}\frac{y_{hj}}{\hat{\sigma}_{hj}^2}}{\sum_{h=1}^{m}\frac{1}{\hat{\sigma}_{hj}^2}}$$

and

$$(A16)\quad \hat{\beta}_j = \frac{\sum_{h=1}^{m}\frac{y_{hj}\hat{P}_{hj}}{\hat{\sigma}_{hj}^2} - \hat{\bar{Y}}_{.j}\sum_{h=1}^{m}\frac{\hat{P}_{hj}}{\hat{\sigma}_{hj}^2} - \hat{\bar{P}}.\sum_{h=1}^{m}\frac{y_{hj}}{\hat{\sigma}_{hj}^2} + \hat{\bar{Y}}_{.j}\hat{\bar{P}}.\sum_{h=1}^{m}\frac{1}{\hat{\sigma}_{hj}^2}}{\sum_{h=1}^{m}\frac{\hat{P}_{hj}^2}{\hat{\sigma}_{hj}^2} - 2\hat{\bar{P}}.\sum_{h=1}^{m}\frac{\hat{P}_{hj}}{\hat{\sigma}_{hj}^2} + \hat{\bar{P}}^2.\sum_{h=1}^{m}\frac{1}{\hat{\sigma}_{hj}^2}}$$

respectively. Similarly the local maximum likelihood estimate for Y_{hj} is y_{hj}. No simple analytical expression for λ_j^2 is attainable by setting Eq. T3 (Table 2) equal to 0. However, if the individual estimation error variances (v_{hj}^2) are ignored such that σ_{hj}^2 is replaced by λ_j^2, then

$$(A17)\quad \hat{\bar{Y}}_{.j} = \bar{y}_{.j},$$

$$(A18)\quad \hat{\beta}_j = \frac{\frac{1}{m}\sum_{h=1}^{m} y_{hj}\hat{P}_{hj} - \hat{\bar{Y}}_{.j}\hat{\bar{P}}.}{\frac{1}{m}\sum_{h-1}^{m}\hat{P}_{hj}^2 - \hat{\bar{P}}^2.},$$

and

$$(A19)\quad \hat{\lambda}_j^2 = \frac{1}{m}\sum_{h=1}^{m}(\hat{\beta}_j\hat{P}_{hj} + (1 - \hat{\beta}_j)\hat{\bar{P}}. + \hat{D}_j - y_{hj})^2 \times \left(\frac{m}{m-2}\right)$$

where the factor $(m/(m-2))$ is a bias correction to adjust the estimate of λ_j^2 for the number of estimated parameters (i.e. degrees of freedom $= m - 2$). Equation A19 includes only the two parameters β_j and $\bar{Y}_{.j}$. The P_{hj}'s enter this likelihood formulation as constants, not parameters, because in this formulation the P_{hj}'s are not statistically evaluated with respect to the sample proportions ($p_h = s_h/n_h$).

Also,

$$(A20)\quad \hat{D}_j = \bar{y}_{.j} - \hat{\bar{P}}. .$$

Appendix B: Solution to Eq. 11

This appendix provides an algorithm to calculate the value of the particular cubic root of Eq. 11 which corresponds to the local maximum likelihood solution of Eq. 10, given estimates for $\bar{Y}_{.j}$, $\bar{P}.$, λ_j^2, and β_j. It can be shown algebraically that the value of P_{hj} yielded by this algorithm is the only one of the three possible roots of Eq. 11 which locally minimizes the negative log-likelihood (i.e., the other two roots correspond to maxima). It can also be shown algebraically that all values for P_{hj} will fall within the domain 0 to 1. The reference for this algorithm is Burlington (1965). Note that the trigonometric operations use radians.

Let Eq. 11 be represented as

$$(B1)\quad 0 = \hat{P}_{hj}^3 + a\hat{P}_{hj}^2 + b\hat{P}_{hj} + c$$

where

$$(B2)\quad a = \left(\frac{\hat{\bar{Y}}_{.j} - y_{hj}}{\hat{\beta}_j}\right) - \hat{\bar{Y}}_{.j} + \hat{D}_j - 1,$$

$$(B3)\quad b = -\left\{\left(\frac{\hat{\bar{Y}}_{.j} - y_{hj}}{\hat{\beta}_j}\right) - \hat{\bar{Y}}_{.j} + \hat{D}_j + \left(\frac{\hat{\sigma}_{hj}^2 n_h}{\hat{\beta}_j^2}\right)\right\},$$

and

$$(B4)\quad c = \frac{\hat{\sigma}_{hj}^2 s_h}{\hat{\beta}_j^2}.$$

From a, b, and c, two new terms, u and v, are defined as

$$(B5)\quad u = \frac{(3b - a^2)}{3}$$

and

$$(B6)\quad v = \frac{(2a^3 - 9ab + 27c)}{27}$$

from which is obtained

$$(B7)\quad \Psi = \arccos\left\{-\operatorname{sign} v\left(\frac{\left(\frac{v^2}{4}\right)}{\left(-\frac{u^3}{27}\right)}\right)^{\frac{1}{2}}\right\}.$$

The desired root of Eq. 11 is

$$(B8)\quad \hat{P}_{hj} = 2\left(-\frac{u}{3}\right)^{\frac{1}{2}}\cos\left(\frac{\Psi + 4\pi}{3}\right) - \left(\frac{a}{3}\right).$$

Appendix C: Bias

We include this appendix to demonstrate that if the individual estimation error variances (v^2_{hj}) are ignored such that σ_{hj}^2 is replaced by λ_j^2, then asymptotically unbiased (i.e. as the values of $n_h \rightarrow \infty$) estimates for all P_{hj}, $\bar{P}_{\cdot}$, β_j, and λ_j^2 are obtainable. If the $v_{hj}{}^2$'s are not ignored, estimates for these parameters will tend toward being unbiased as the values for the $v_{hj}{}^2$'s become increasingly small relative to λ_j^2.

An estimator (e.g. $\hat{\Omega}$) for any parameter ω is said to be unbiased if the expected value of the estimator $E[\hat{\Omega}]$ equals the true value (ω) of the parameter, i.e. $E[\hat{\Omega}] = \omega$. We shall let $\tilde{\omega}$ represent an unbiased estimate of ω.

We start by recognizing from Eq. 5 that

$$\text{(C1)} \quad E[y_{hj}] = Y_{hj}.$$

Recalling that the local maximum likelihood estimate of $\hat{Y}_{hj}$ is y_{hj}, we have

$$\text{(C2)} \quad \tilde{Y}_{hj} = y_{hj}$$

and consequently

$$\text{(C3)} \quad \tilde{\bar{Y}}_{\cdot j} = \bar{y}_{\cdot j}\,.$$

Representing Eq. 11, which is an estimator for the P_{hj}'s, as

$$\text{(C4)} \quad 0 = E\left[- \hat{P}_{hj}^3 - \left\{ \left(\frac{\hat{\bar{Y}}_{\cdot j} - y_{hj}}{\hat{\beta}_j} \right) - \hat{Y}_{\cdot j} + \hat{D}_j - 1 \right\} \hat{P}_{hj}^2 \right]$$

$$+ E\left[\left\{ \left(\frac{\hat{\bar{Y}}_{\cdot j} - y_{hj}}{\hat{\beta}_j} \right) - \hat{\bar{Y}}_{\cdot j} + \hat{D}_j + \left(\frac{\hat{\lambda}_j^2 n_h}{\hat{\beta}_j^2} \right) \right\} \hat{P}_{hj} - \left\{ \frac{\hat{\lambda}_j^2 s_h}{\hat{\beta}_j^2} \right\} \right]$$

and knowing that s_h is a binomial random variable with an expected value of $n_h P_{hj}$, i.e.

$$\text{(C5)} \quad E[s_h] = n_h P_{hj}\,,$$

we then have

$$\text{(C6)} \quad P_{hj} = E\left[\frac{\hat{\beta}_j^2}{\hat{\lambda}_j^2 n_h} \right] \times E\left[- \hat{P}_{hj}^3 - \left\{ \left(\frac{\hat{\bar{Y}}_{\cdot j} - y_{hj}}{\hat{\beta}_j} \right) - \hat{\bar{Y}}_{\cdot j} \right.\right.$$

$$\left.\left. + \hat{D}_j - 1 \right\} \hat{P}_{hj}^2 + \left\{ \left(\frac{\hat{\bar{Y}}_{\cdot j} - y_{hj}}{\hat{\beta}_j} \right) - \hat{\bar{Y}}_{\cdot j} + \hat{D}_j + \left(\frac{\hat{\lambda}_j^2 n_h}{\hat{\beta}_j^2} \right) \right\} \hat{P}_{hj} \right].$$

It can be shown easily that in the limit as $n_h \rightarrow \infty$, Eq. C6 simplifies to $E[\hat{P}_{hj}] = P_{hj}$, thus confirming that $\hat{P}_{hj}$, and consequently $\hat{\bar{P}}_{\cdot}$, are asymptotically unbiased.

It follows from Eq. C2, C3, and C6 that in the limit as the n_h's $\rightarrow \infty$

$$\text{(C7)} \quad \tilde{\beta}_j = \frac{\frac{1}{m} \sum_{h=1}^{m} \tilde{Y}_{hj} \hat{P}_{hj} - \hat{\bar{Y}}_{\cdot j} \hat{\bar{P}}_{\cdot}}{\frac{1}{m} \sum_{h-1}^{m} \hat{P}_{hj}^2 - \hat{\bar{P}}_{\cdot}^2}$$

and

$$\text{(C8)} \quad \tilde{\lambda}_j^2 = \frac{1}{m} \sum_{h=1}^{m} (\hat{\beta}_j \hat{P}_{hj} + (1 - \hat{\beta}_j) \hat{\bar{P}}_{\cdot}$$

$$+ \hat{D}_j - y_{hj})^2 \times \left(\frac{m}{m-2} \right).$$

We thus conclude that in the limit as the n_h's $\rightarrow \infty$ the binomial–normal functional model becomes equivalent to ordinary regression.

Food Consumption of Juvenile Coho (*Oncorhynchus kisutch*) and Chinook Salmon (*O. tshawytscha*) on the Continental Shelf off Washington and Oregon

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Brodeur, R. D., R. C. Francis, and W. G. Pearcy. 1992. Food consumption of juvenile coho (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*) on the continental shelf off Washington and Oregon. Can. J. Fish. Aquat. Sci. 49: 1670–1685.

The overall food consumption by juvenile coho (*Oncorhychus kisutch*) and chinook salmon (*O. tshawytscha*) along the continental shelf off Washington and Oregon was estimated for the summer months of 1981–84 using both a direct field and indirect bioenergetic approach. Both models yielded similar estimates but the consumption estimated by the bioenergetic model was 14.8 and 3.2% higher for coho and chinook salmon, respectively. In a given year, overall consumption increased throughout the summer, despite decreasing abundances of both salmon species, but there were substantial differences among the four years within the same month. A preliminary comparison of the consumption estimates for important prey taxa early in the summer with estimated zooplankton biomass suggested that sufficient food levels may have been available for salmon during 1981. During the El Niño of 1983, however, several prey groups were consumed at high levels relative to their estimated availability and the biomass of these taxa may not have been sufficient to sustain the total population of salmon.

À partir de travaux sur le terrain et d'études sur la bioénergétique menées en laboratoire, on a effectué une estimation de la quantité globale de nourriture consommée pendant les mois d'été de 1981 à 1984 par les juvéniles du saumon coho (*Oncorhynchus kisutch*) et du saumon quinnat (*O. tshawytscha*) fréquentant les eaux de la plate-forme continentale bordant les États de l'Oregon et de Washington. Les deux modèles ont donné des estimations semblables bien que la quantité de nourriture consommée par le saumon coho et le saumon quinnat, telle que déterminée à partir du modèle bioénergétique, était respectivement 14,8 et 3,2 % plus élevée. Au cours d'ue année particulière la quantité globale de nourriture consommée a augmenté tout au cours de l'été malgré une baisse de l'abondance des deux espèces de saumon, mais il existait des différences nettes entre le même mois des quatre années de l'étude. Une comparaison préliminaire des estimations de la quantité des principales proies consommées au début de l'été et de la biomasse zooplanctonique estimative indique que le saumon pouvait disposer de quantités suffisantes de nourriture en 1981. Par contre, lors de l'apparition du El Niño en 1983, plusieurs groupes de proies ont été consommés à des taux élevés par rapport à leur niveau de disponibilité estimatif; de plus, la biomasse de ces taxons peut ne pas avoir suffit à nourrir toute la population de saumon.

Received February 7, 1991
Accepted February 14, 1992
(JA889)

Reçu le 7 février 1991
Accepté le 14 février 1992

A basic premise of salmonid enhancement programs is that the fundamental limitation to Pacific salmon (*Oncorhynchus* spp.) production occurs mainly in freshwater and that by increasing the quantity and quality of smolts released, substantial gains in adult returns can be realized. The ocean environment has been assumed to be nonlimiting because historical wild salmon abundances were much higher than the combined abundances of wild and hatchery salmon at present (Chapman 1986; Bledsoe et al. 1989) and standing stocks and production rates of prey resources were estimated to far exceed the food requirements of the present ocean populations (LeBrasseur 1972; Sanger 1972).

A number of studies have found evidence that ocean conditions limit salmon production and that a substantial percentage of the total natural mortality may occur during early marine life (Parker 1968; Mathews and Buckley 1976; Bax 1983; Furnell and Brett 1986; Fisher and Pearcy 1988). In many populations, much of this mortality appears to occur in the first month at sea, regardless of the number of smolts released.

Some recent studies (Brodeur and Pearcy 1990; Brodeur 1991) suggest that early ocean feeding may be important, and perhaps critical, to the survival of juvenile coho (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*). These studies have shown that the diets of both species differ substantially among years, months, and size of predator and to a lesser extent among latitudinal strata and inshore–offshore strata. Substantial variability in feeding intensity was also observed depending on date and location of sampling (Brodeur 1992).

Data on variation in diet of juvenile salmon will be used here as a starting point to estimate food consumption by coho and chinook salmon during their first summer in the ocean. These estimates will then be compared with estimates of zooplankton

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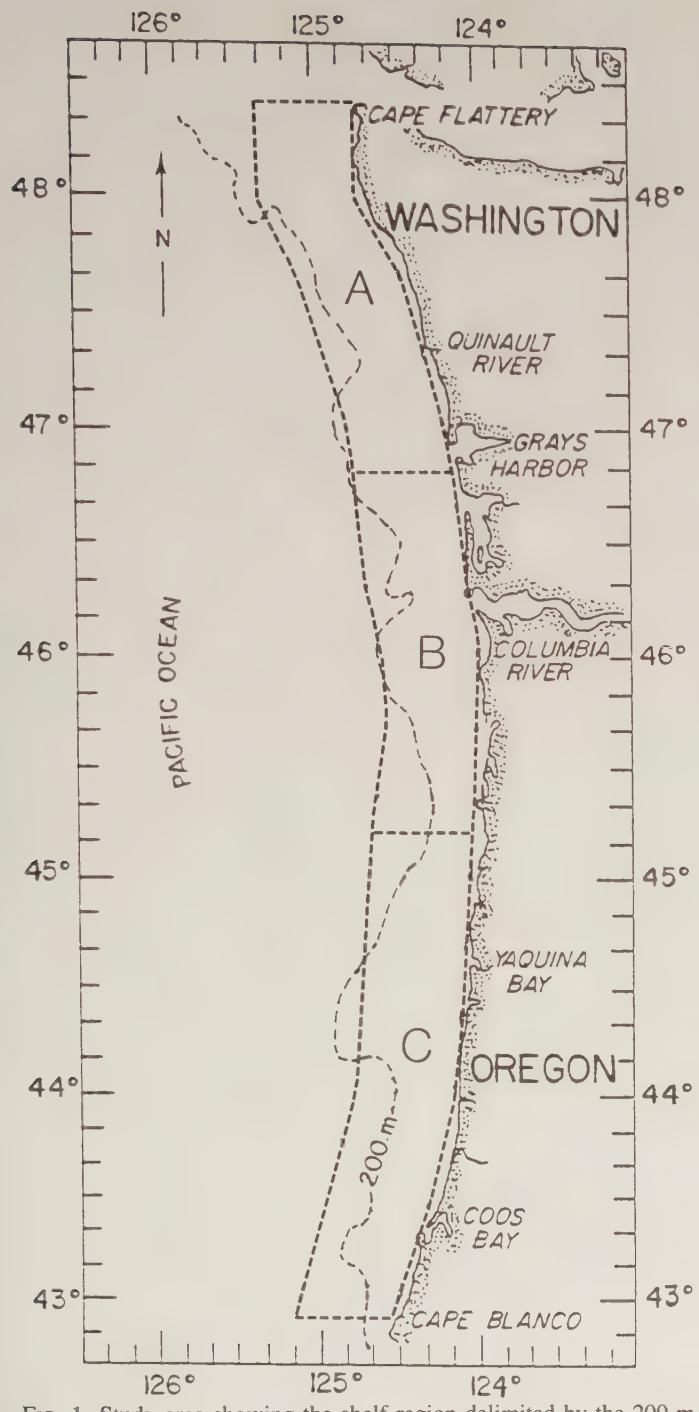


FIG. 1. Study area showing the shelf region delimited by the 200-m isobath (light broken lines) and the three geographic subareas (A = Washington, B = Columbia, C = Oregon) used in this study (heavy broken lines).

standing stocks as a way of examining the carrying capacity of the coastal environment. The one previous attempt to examine the coastal carrying capacity for juvenile salmonids occurring off British Columbia using a simulation model (Walters et al. 1978) did not provide any evidence for food limitation. This model, however, lacked data on several key aspects of the biology of salmon during their early marine life. Data are now available on many of these (e.g. food habits, daily ration, growth rates) and will be used in this paper to estimate the food consumption of juvenile coho and chinook salmon on the continental shelf off Washington and Oregon during 1981–84.

Two independent estimates of food consumption will be derived in this analysis. The first is based on field measurements of stomach fullness over the diel period and laboratory measurements of gastric evacuation rates. These were combined to estimate a daily ration (Brodeur and Pearcy 1987) which is then expanded to the entire population. The second method is based on indirect bioenergetic modelling which estimates the consumption necessary to achieve the observed growth rates at the observed temperatures (Hewett 1989; Ney 1990).

Methods

Salmon Abundance Estimates

The abundance of juvenile salmon in each year–month–area stratum was estimated from quantitative purse seine collections made during 1981–84 off Oregon and Washington (see Pearcy and Fisher (1988) for collection details). All quantitative sets made within 46 km of shore were grouped into one of three geograhic areas (Fig. 1) and an average catch per set ($\bar{C}_i$) was calculated for each area. Repeat sets at the same location were averaged and included only once. A population estimate ($\hat{P}$) of the total number of juvenile salmon in the coastal zone was calculated according to the formula of Cochran (1977):

$$\hat{P} = \sum_{i=1}^{3} \frac{A_i}{a} \bar{C}_i$$

where A_i = area of stratum i and a = area sampled in one set.

Due to the lognormal distributions of the catches per set (Pearcy and Fisher 1990), the catch per set of juvenile coho and chinook salmon in each subarea by month was ln $(x + 1)$ transformed so that the assumptions of normality were met. Estimates for the 95% confidence limits of $\hat{P}$ can be obtained by

$$\hat{P} \pm \mathrm{t}\,(0.95, n_e)\,(\mathrm{Var}\,\hat{P})^{1/2}$$

where

$$\mathrm{Var}\,\hat{P} = \sum_{i=1}^{3} \left(\frac{A_i}{a}\right)^2 \mathrm{Var}\,\bar{C}_i$$

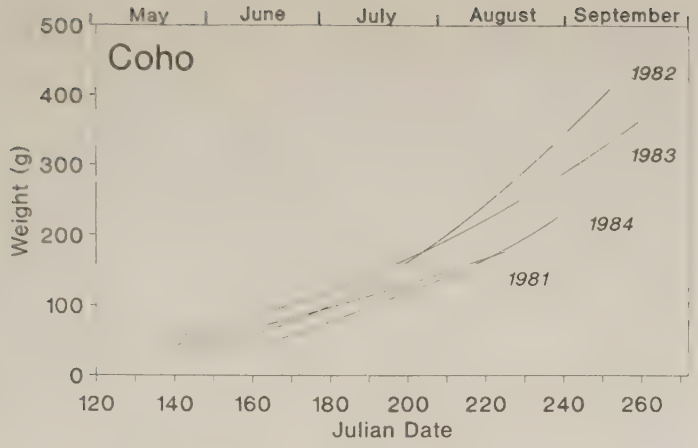
$$\mathrm{Var}\,\bar{C}_i = \frac{1}{n_i\,(n_i - 1)} \sum_{j=1}^{n_i} (C_{ij} - \bar{C}_i)^2$$

where n_e = effective degrees of freedom (Cochran 1977).

The estimated abundance in each stratum was then expanded to the estimated total number of salmon in each area based on the proportion of the total area sampled during the surveys.

Food Habits Analysis

The proportion by weight of each major prey category consumed in each year–month–area subset was estimated from food habits data examined for each subset (see Brodeur and Pearcy (1990) for sampling methods). Quantitative food habits data were available from a total of 1465 juvenile coho and 756 juvenile chinook salmon collected from 1981 to 1984. Variation in diet attributable to predator size (Brodeur 1991) was not explicitly represented in our food consumption model but size variation is directly related to the time of year, which was included in the analysis.



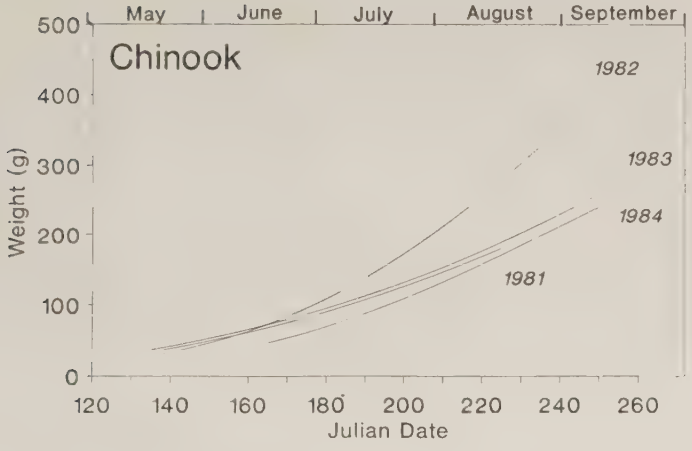
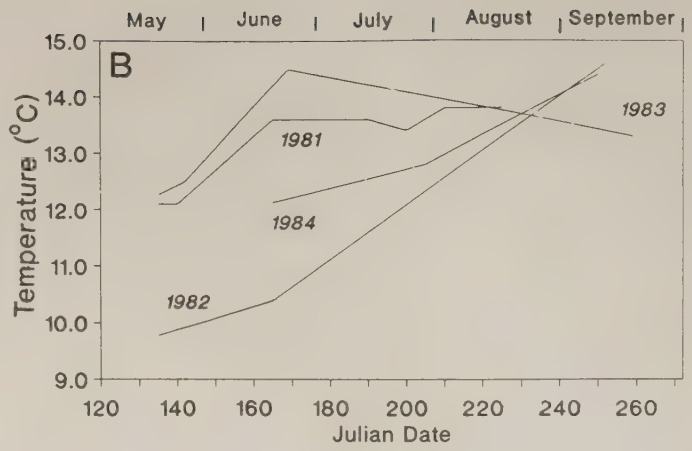


FIG. 2. Simulated growth curves for juvenile coho and chinook salmon generated by the bioenergetic model for each year.



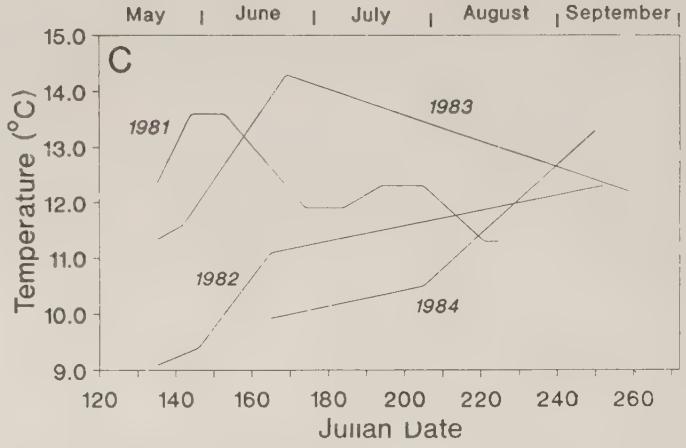


FIG. 3. Simulated temperature cycle at 10 m depth for area B (Columbia) and area C (Oregon) generated by the bioenergetic model for each year.

Daily Ration Estimates

The daily ration of coho salmon was estimated using both field and laboratory data (Brodeur and Pearcy 1987). Measurements were made of the stomach content weight of juvenile coho salmon collected throughout the diel period in the field. Estimates were made of the gastric evacuation rate at two temperatures (11.3 and 13.7°C) which roughly correspond to early (May and June) and late summer (July–September) water temperatures off Oregon and Washington (Brodeur and Pearcy 1987). The field and laboratory data were used together to calculate daily ration utilizing the method of Elliott and Persson (1978). Daily ration estimates were not made for chinook salmon due to lack of fish collected during the nighttime sets but we have assumed the same daily ration for chinook that was used for coho salmon at comparable fish weights and temperatures. The daily ration estimates in percent body weight consumed per day were converted to mean biomass per fish by multiplying them by the mean weights of juvenile salmon in each year–month subset given in Fisher and Pearcy (1988) for coho salmon and J. Fisher (College of Oceanography, Oregon State University, Corvallis, OR, pers. comm.) for chinook salmon. Daily ration estimates were adjused for differences in mean temperatures among the year–month–area strata by linear interpolation.

Direct Food Consumption Model

The consumption of eight major prey categories was estimated separately and then summed to get an estimate of the total food consumed by juvenile salmon in grams for each year, month, and area. The following model was used:

$$\text{Total consumption} = \sum_{l=1}^{8} C_{ijkl} = \sum_{l=1}^{8} A_{ijk} \cdot P_{ijkl} \cdot R_{ij} \cdot D_j$$

where A_{ijk} = abundance of juvenile salmon in the ith year, jth month, and kth area, P_{ijkl} = proportion of diet by weight of prey l in the ith year, jth month, and kth area, R_{ij} = daily ration in the ith year and jth month, D_j = number of days in the monthly period, i = years (1981, 1982, 1983, 1984), j = months (May, June, July–August, September), k = areas (Washington, Columbia River, Oregon), and l = prey category (fishes, decapod larvae, euphausiids, amphipods, pteropods, copepods, insects, other taxa).

Consumption estimates were made for each subset using the estimated geometric mean population level ($\hat{P}$) and the upper and lower 95% confidence intervals to represent medium, high, and low population abundances, respectively. Consumption of each prey was first standardized to grams consumed per square kilometre of sea surface area. The overall consumption in grams

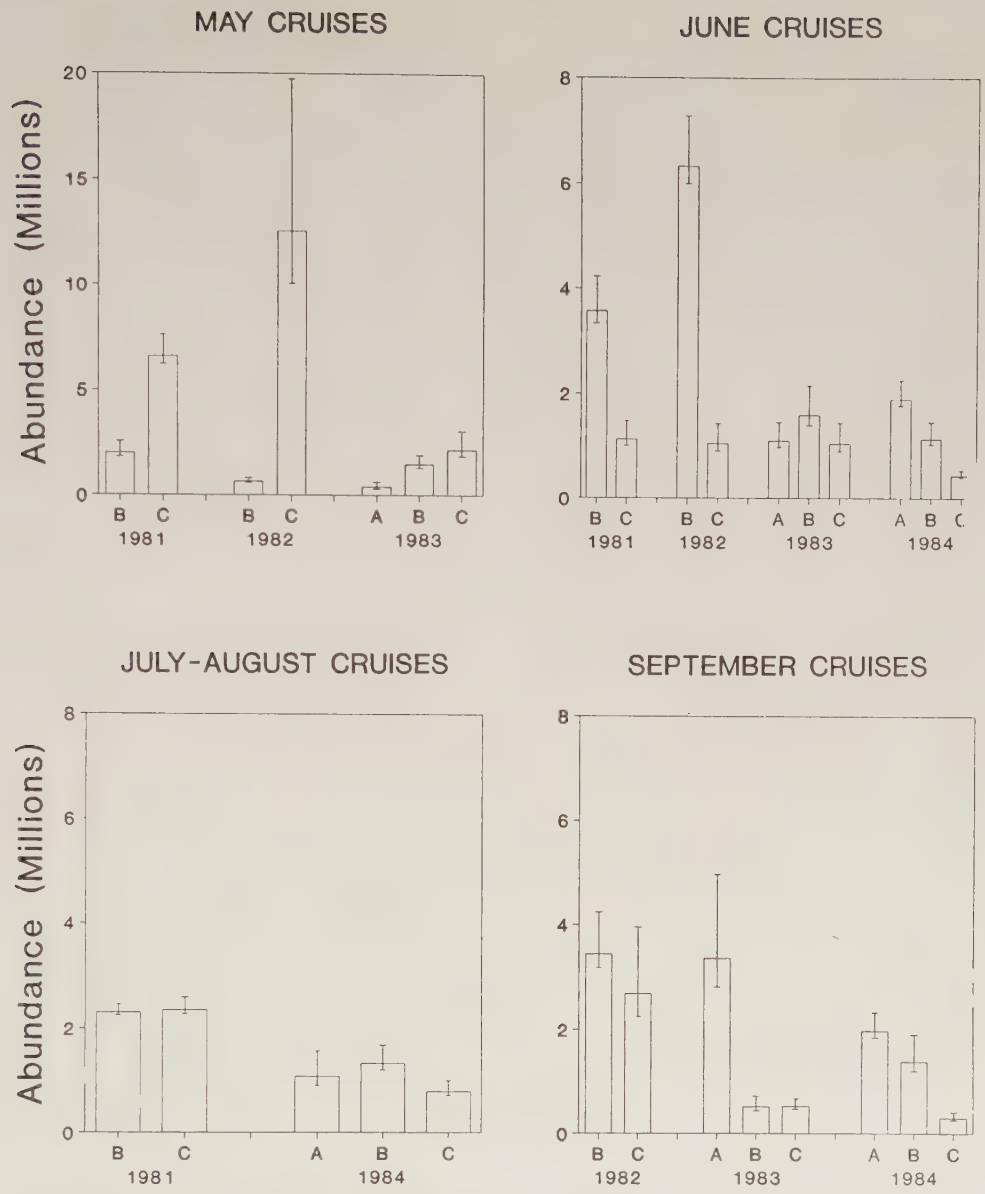


FIG. 4. Geometric mean abundance (bars) and 95% confidence interval about the mean (error bars) abundance of juvenile coho salmon for each month, year, and subarea. Only areas B and C were sampled during 1981 and 1982. Note the asymmetric error bars resulting from the backtransformation of the logged abundances.

for each area was then multiplied by a scaling factor to express the consumption in metric tons for the total area in a stratum.

Bioenergetic Model

Food consumption estimates for juvenile coho and chinook salmon were also made using the balanced energy equation (Kitchell 1983; Ney 1990)

$$\text{Growth} = \text{Consumption} - (\text{Respiration} + \text{Egestion} + \text{Excretion}).$$

Individual marine growth rates in grams per day are available from recoveries of coded-wire tagged coho (Fisher and Pearcy 1988) and chinook salmon (W. Pearcy, unpubl.) taken in these purse seine collections. These were averaged by cruise month to arrive at an overall mean weight for each month, assuming that there were no differences in growth rate between tagged and untagged fish. Growth curves were forced through these observed monthly mean weights by the bioenergetic model for both species and were then used as the mean population growth rate (Fig. 2).

We ran computer simulations of the daily population consumption of the prey categories listed above using the microcomputer-based program of Hewett and Johnson (1987). The required physiological parameters for salmon have been estimated in numerous studies (see Brett and Groves 1979) and are available for both species of concern in this study (Stewart and Ibarra 1991). The Thorton and Lessem (1978) algorithm was used to adjust for temperature dependence of maximum food consumption.

Since both species tend to be found much of the day within the upper 20 m of the water column (Pearcy and Fisher 1988; W. Pearcy, unpubl.), we averaged the temperatures measured by CTD at 10 m for each year–month–area subset. These tended to be several degrees centigrade below the surface temperatures

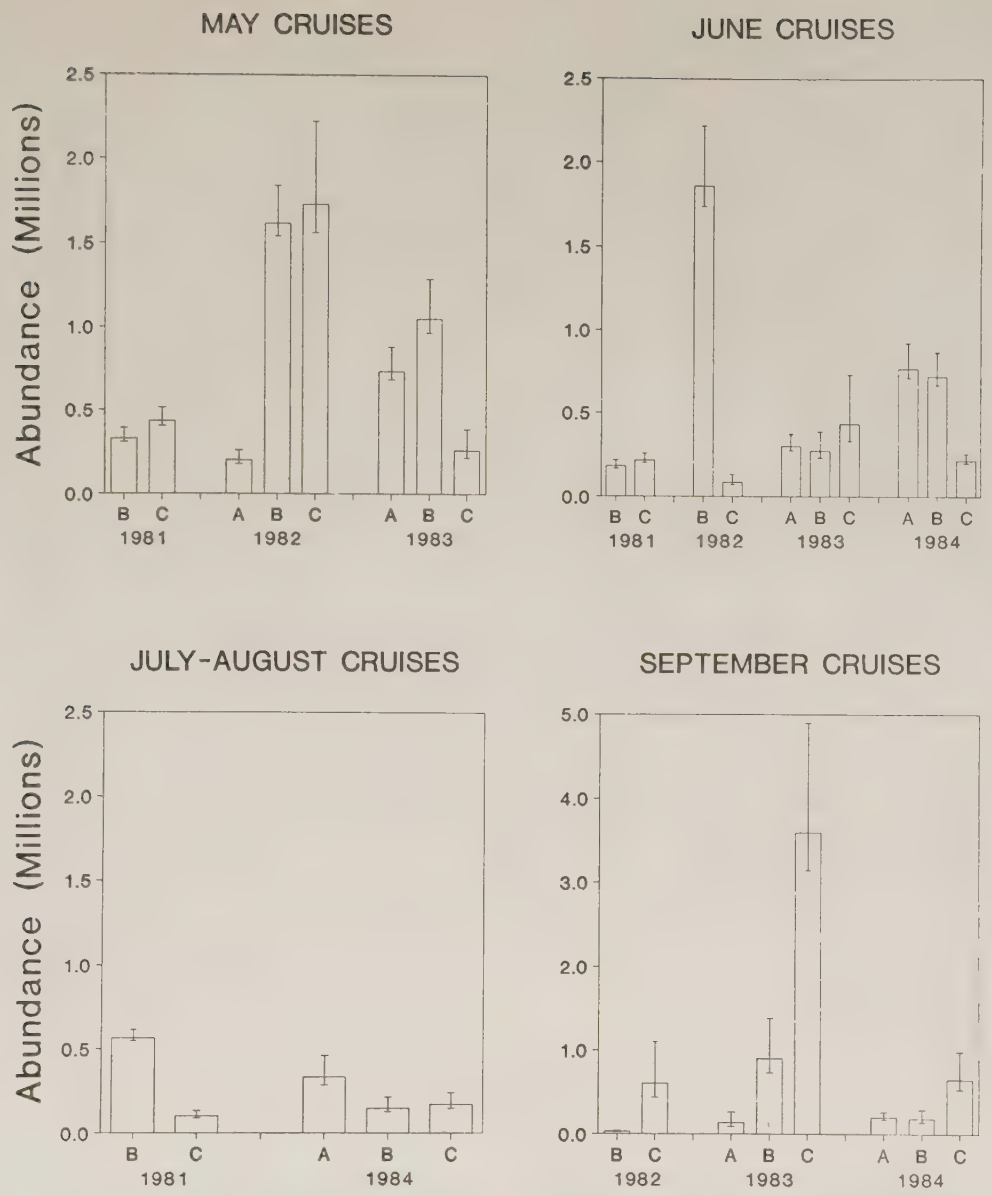


FIG. 5. Geometric mean abundance (bars) and 95% confidence interval about the mean (error bars) abundance of juvenile chinook salmon for each month, year, and subarea. Only areas B and C were sampled during 1981 and 1982. Note the asymmetric error bars resulting from the backtransformation of the logged abundances.

(Brodeur and Pearcy 1992). Since CTD measurements were not available for 1981, we estimated these by applying a correction factor to the surface temperatures based on the relationships for the other three years. We used interpolation routines in the bioenergetic program to simulate the temperature cycle throughout the whole summer. Examples of these simulated temperature cycles for the Columbia and Oregon subareas for each year are shown in Fig. 3.

The proportion of the diet made up by the major prey categories was entered into each simulation at the midpoint of each cruise period. The program interpolated between these midpoints to estimate the seasonal cycle of prey consumption. Energy densities of prey were derived either from literature values (Cummins and Wuycheck 1971; Nishiyama 1977; Steimle and Terranova 1985) or, as in the case of some decapod larvae and amphipods, from combusting triplicate samples of previously frozen zooplankton samples in a Parr adiabatic bomb calorimeter. Caloric values of the predators were available from Ruggerone and Rogers (1992) and Stewart and Ibarra (1991).

Simulations were run on a daily time step over the part of the summer sampled each year starting on May 15 as day 0 for 1981–83 and June 15 as day 0 for 1984. Individual consumption generated by the model for day 16 of each month was used as the consumption rate for that month and then expanded to the whole population sizes for that month as estimated above. The only exception to this was that individual consumption on August 1 was used for the July–August period.

Zooplankton Biomass Estimates

Data on macrozooplankton biomass are available only for the 1981 cruises. A complete description of the methods for collection and analysis of the zooplankton is given in Brodeur (1990). Briefly, stepped oblique 70-cm bongo net tows were made from surface to near bottom at 86 stations off Oregon and

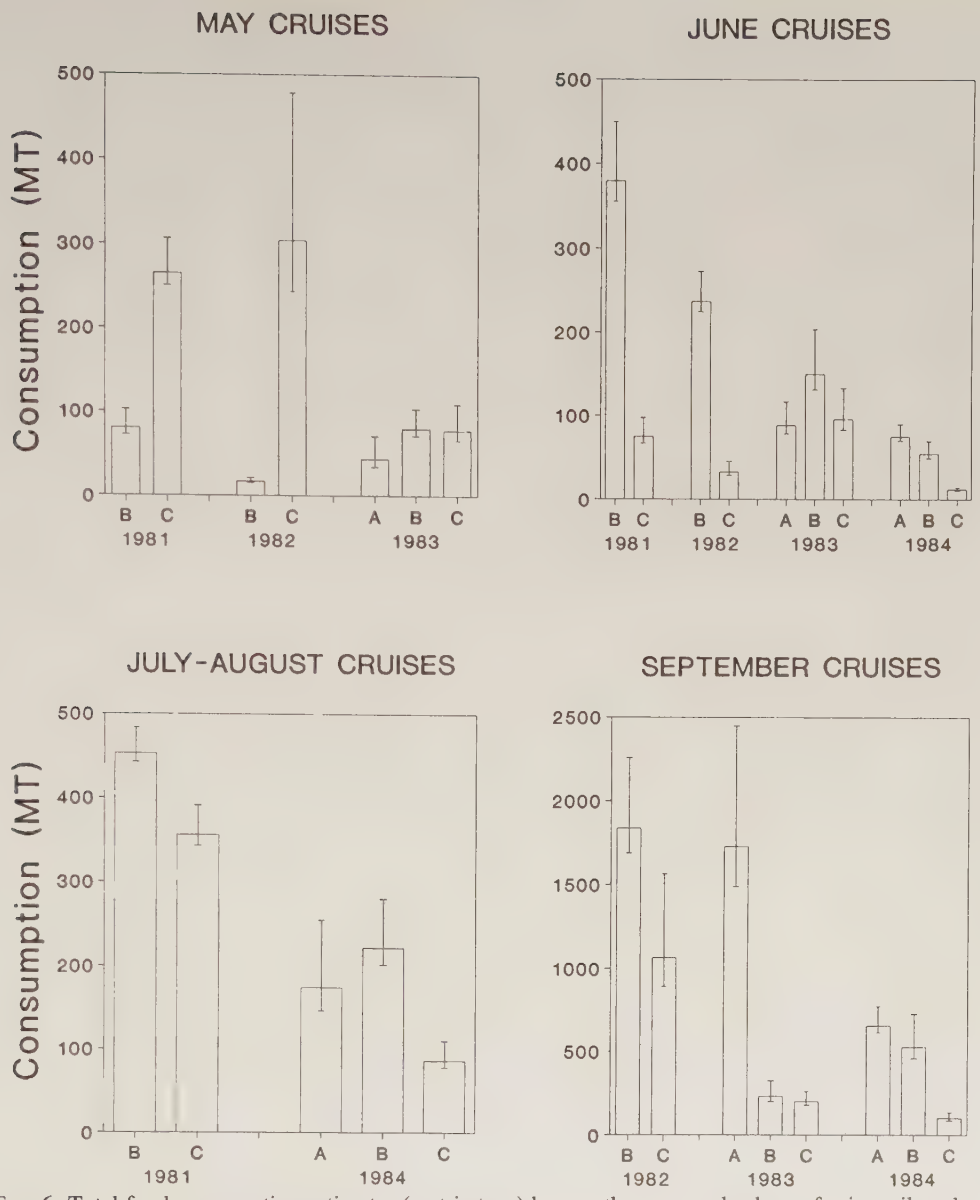


FIG. 6. Total food consumption estimates (metric tons) by month, year, and subarea for juvenile coho salmon. The bars represent consumption at the geometric mean abundance level and the error bars represent consumption at the upper and lower 95% population estimates.

southern Washington from May through August of 1981. Collections from one side of the bongo containing a 571-μm-mesh net were sorted into major taxonomic categories and blotted damp weights were taken for each of these categories. These were expressed as grams wet weight per 10 m^2 of sea surface area and then converted to grams per square kilometre and averaged over each sampling area so that comparisons could be made with the field consumption estimates.

Results

Salmon Abundance

Juvenile coho and chinook salmon mean population estimates and 95% confidence intervals are available for all four years of June cruises, three years of May and September cruises, and two years of July–August cruises. The survey sampled in total only the Columbia (area B) and Oregon (C) regions during 1981 and 1982.

Coho salmon abundance was generally much higher in areas B and C during 1981 and 1982 than during 1983 and 1984, when the abundance was uniformly low (Fig. 4). The most northern region off Washington (A) in September of 1983 was the only subset in these two years which showed moderately high abundance of coho salmon. The overall abundance of coho salmon declined as the summer progressed during each year and there was a general northward shift in the abundance peak from area C (Oregon) in May to area A (Washington) in September.

Overall, chinook salmon abundance levels were generally much lower than those found for coho salmon within a given cruise, with the exception of September of 1983 when they were similar (Fig. 5). The abundance of chinook salmon juveniles did not show the same interannual or seasonal patterns as seen for coho salmon juveniles. The May and June cruises during 1983 showed higher abundance than these same months during 1981. The overall abundance remained high in July-August and September, possibly due to an influx of subyearling

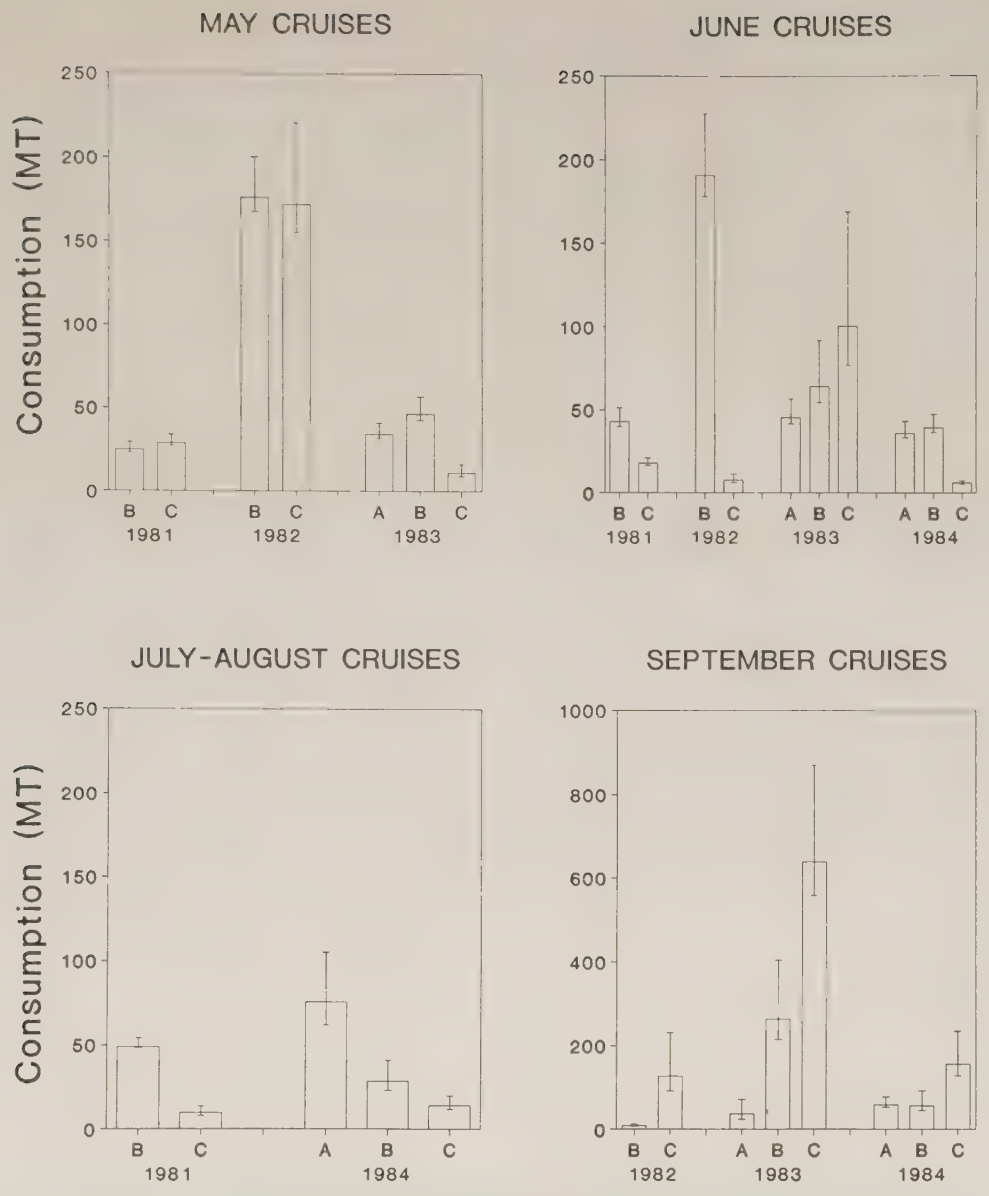


FIG. 7. Total food consumption estimates (metric tons) by month, year, and subarea for juvenile chinook salmon. The bars represent consumption at the geometric mean abundance level and the error bars represent consumption at the upper and lower 95% population estimates.

fish in midsummer. Chinook salmon abundances were high in the Columbia area (B) in June during 1982 and off Oregon (C) in September during all years.

Total Food Consumption

Overall monthly food consumption by juvenile coho salmon estimated by the direct field method within each area increased as the summer progressed during all years (Fig. 6). This was due in part to the elevated temperatures present later in the year which led to a substantial increase in daily ration per individual fish and also a larger mean size of fish despite a decline in total abundance. Consumption in 1981 and 1982 was highest off Oregon (C) during May but was generally much higher in the Columbia region (B) during June. Higher overall consumption off Oregon, however, may reflect the greater total area (8450 km^2) included in this region relative to that of the Columbia region (5850 km^2). Although overall consumption was higher within an area in 1981 and 1982 than in 1983 and 1984, the difference was not as large as would be expected based on differences in the population estimates (Fig. 4) due to the higher water temperatures occurring during the El Niño of 1983–84. An exception to this was the high levels of consumption taking place during September of 1983 off Washington (area A), where the highest abundance was found.

Overall consumption by juvenile chinook salmon increased substantially throughout the summer only during 1983 and 1984 and remained level (1981) or apparently declined (1982) during the other years (Fig. 7). As found for coho salmon, consumption increased dramatically during September, but unlike coho, the consumption by chinook salmon was highest in the southernmost subarea (C).

Comparison with Bioenergetic Estimates

Total food consumption estimates derived from the bioenergetic model were very similar to those estimated from the field (Fig. 8). The bioenergetic model tended to produce higher esti-

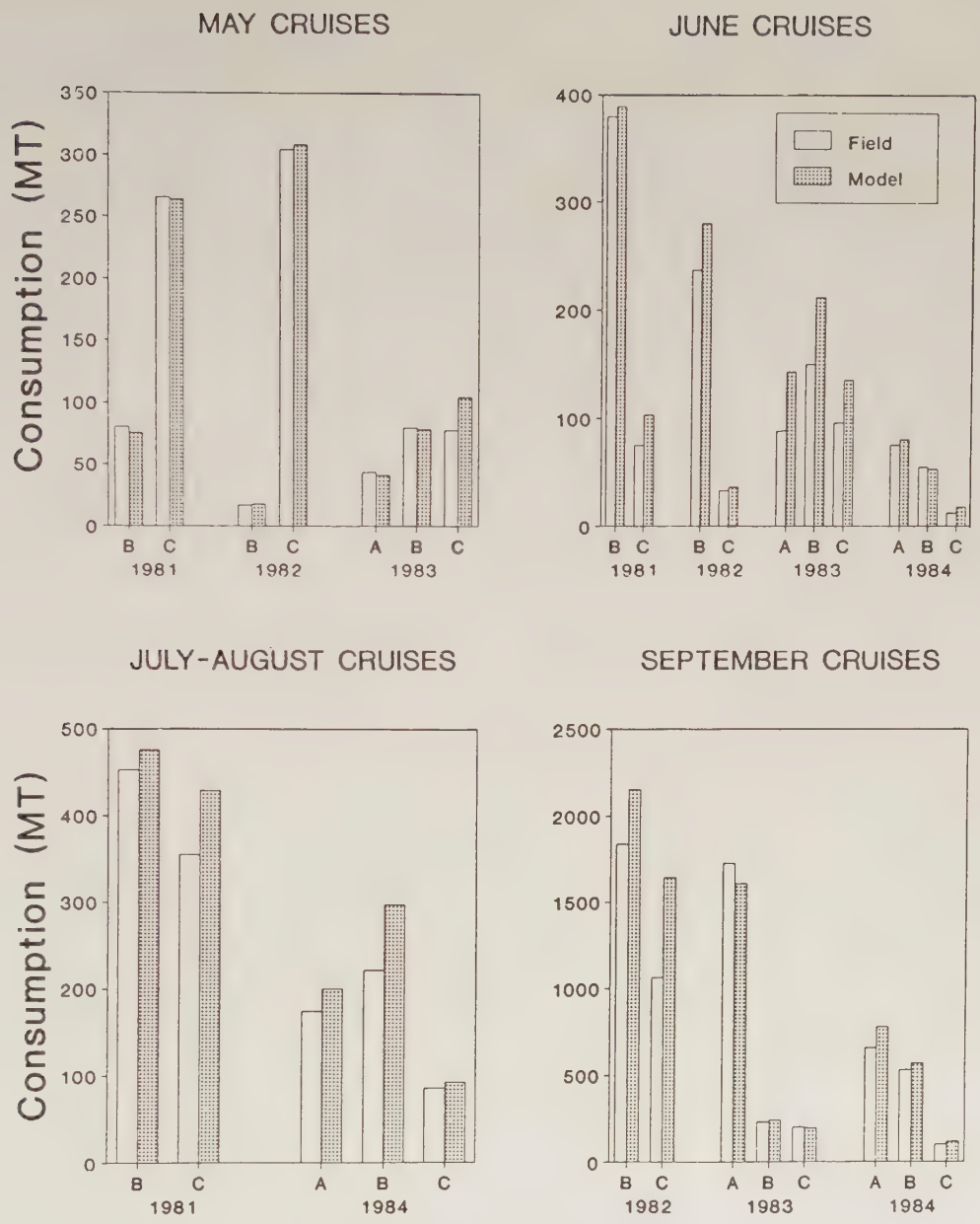


FIG. 8. Comparison of the total consumption (metric tons) estimated by the field and bioenergetic methods at the geometric mean population sizes of coho salmon.

mates of consumption, especially during the warm years (1983 and 1984) or months (July–August through September). The absolute values of the percentage differences between the two models ranged from 0.6 to 61.4 (mean 17.9) for the different year–month–area subsets and the overall difference in total consumption for all subsets combined,

$$\frac{|\Sigma\,(\text{Bioenergetic}) - \Sigma\,(\text{Field})|}{\Sigma\,(\text{Field})} \cdot 100,$$

was only 14.8%. The consumption estimates for chinook salmon by the two methods were also similar but the field method tended to produce higher estimates of consumption than the bioenergetic method more often than for coho salmon juveniles (Fig. 9). The absolute values of the percent differences ranged from 1.6 to 77.9 (mean = 20.9) but because higher consumptions were found for each method depending on the subset, the overall consumption difference was only 3.2%.

Food Consumption by Taxonomic Category

The total food consumed by juvenile coho salmon by both methods was partitioned into major taxonomic categories based on the diets obtained within each year–month–area subset and then standardized to daily consumption per square kilometre of sea surface area for average abundance levels (Tables 1 and 2). Fish prey made up the majority of food during every year–area subset of May and June with the exception of area C (Oregon) during June of 1984, when euphausiids were equally important. Prey other than fish (e.g. euphausiids, pteropods, insects, and amphipods) were relatively more important during July–August. A much higher biomass of fish was consumed during September overall compared with the other months but within some subsets, euphausiids, hyperiid amphipods, and pteropods were also important.

For chinook salmon, the number of stomachs examined was insufficient to adequately describe the diet for several subsets;

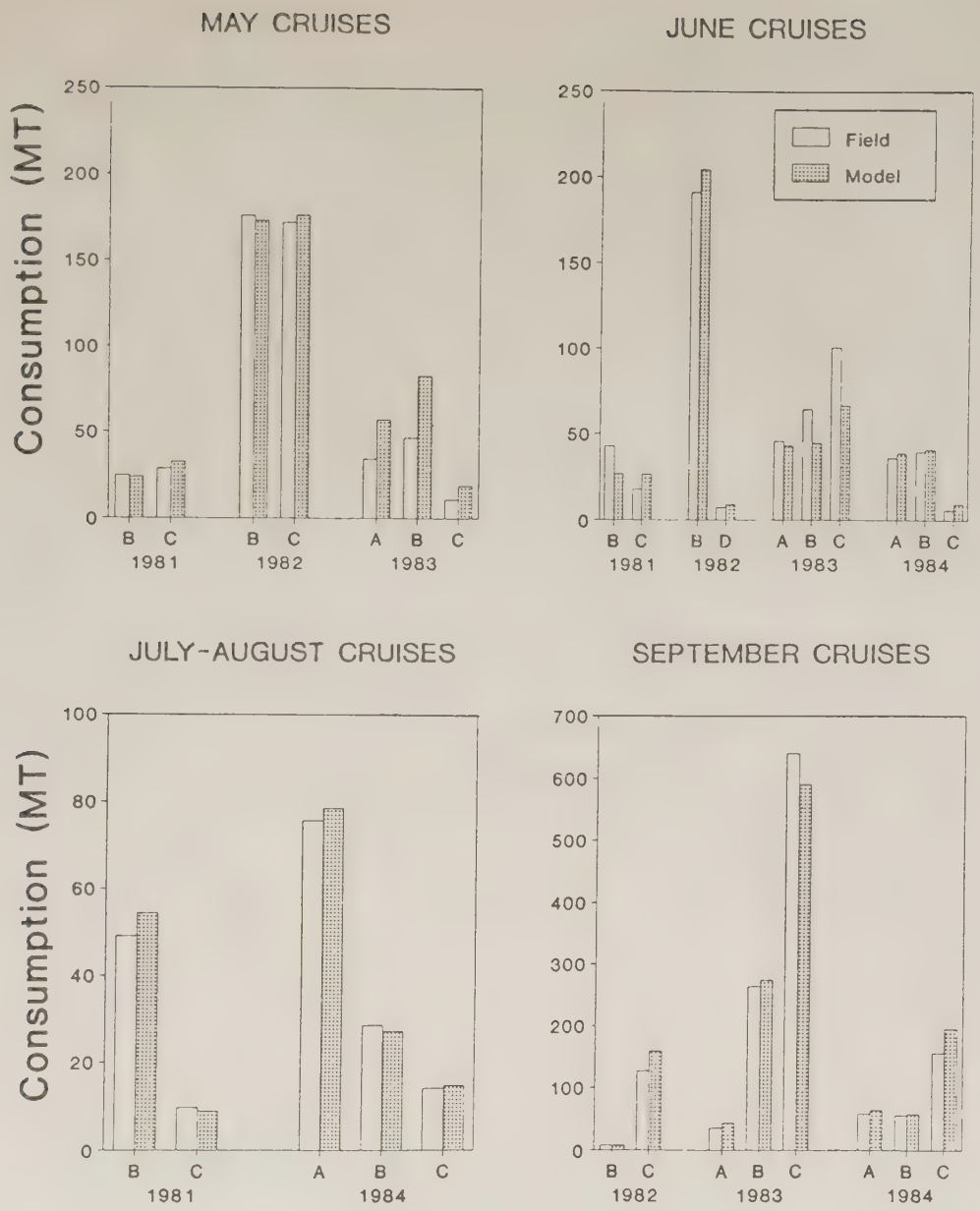


FIG. 9. Comparison of the total consumption (metric tons) estimated by the field and bioenergetic methods at the geometric mean population sizes of chinook salmon.

therefore the total food was not partitioned into separate prey categories in these subsets (Tables 3 and 4). Total consumption by chinook salmon juveniles was generally lower than that of coho salmon juveniles for all prey categories. Fish were again the major prey consumed within every subset except for June of 1984, when euphausiids were predominant.

Comparison with Plankton Biomass

Euphausiids were the dominant taxonomic category collected during all cruises (Table 5). Chaetognaths made up a large proportion of the biomass in July and August, although they were not a major prey of coho and chinook salmon juveniles. Fishes were the dominant food consumed by juvenile salmon but made up a relatively minor proportion of the plankton biomass. This suggests that fishes are either consumed in higher proportion relative to their abundance or that the sampling gear used was not effective in capturing juvenile fishes and that their biomass is much higher than that estimated.

Estimated zooplankton biomasses were compared with the total coho and chinook salmon consumption estimated by the field method for each time period (both areas combined) during 1981 to estimate what percentage of the total available plankton standing stocks was consumed at the average and maximum estimated salmon abundances (Table 6). Salmon consumption as a percentage of total biomass was low (0.04–0.08%·d^{-1} for average abundances and 0.05–0.10%·d^{-1} for maximum abundances) for all time periods. When examined by individual prey taxa, however, some relatively high utilization rates were found for fish (2.4–6.1 and 2.7–6.7%·d^{-1} for average and maximum abundances, respectively) and pteropods (16.0–17.6%·d^{-1} in July–August).

Discussion

Our calculations, although subject to some caveats based on the sampling limitations and several critical assumptions, represents, nonetheless, the most complete attempt at estimating

TABLE 1. Daily consumption ($g \cdot km^{-2}$) of the major taxonomic groups by year–month–area subsets for average population sizes of juvenile coho salmon by the field method. Dashes indicate that those prey were not found in that particular subset; tr = $<0.1\ g \cdot km^{-2}$.

Year	Area	Fish	Decapods	Euphausiids	Amphipods	Pteropods	Insects	Copepods	Other	Total
					May					
1981	B	39.6	2.4	0.2	0.1	0.4	0.3	0.1	1.1	44.2
	C	53.7	15.0	3.3	1.2	18.7	—	0.2	9.1	101.3
1982	B	5.9	0.4	2.6	tr	—	tr	—	0.5	9.4
	C	87.1	12.2	10.5	2.1	0.6	—	0.2	3.5	116.1
1983	A	14.3	4.9	0.3	0.1	1.4	—	—	0.8	21.8
	B	32.8	2.5	3.6	1.5	tr	0.3	1.3	1.9	44.0
	C	22.1	1.7	2.5	1.0	tr	0.2	0.9	1.3	29.6
					June					
1981	B	172.3	12.5	21.2	0.9	3.5	—	0.9	5.0	216.2
	C	21.3	5.6	1.5	0.1	0.2	tr	tr	0.7	29.6
1982	B	89.4	9.0	15.5	10.5	0.3	0.8	0.1	9.3	135.0
	C	11.3	tr	1.2	tr	—	—	—	0.5	13.0
1983	A	35.1	8.6	0.1	0.2	—	0.1	0.1	1.0	45.3
	B	66.8	12.7	0.6	2.8	—	—	0.1	2.4	85.5
	C	32.2	2.0	1.5	1.3	—	tr	—	0.9	37.9
1984	A	31.1	5.3	2.1	1.1	—	—	tr	tr	39.5
	B	30.6	0.6	tr	—	—	—	—	tr	31.3
	C	2.4	0.3	2.1	tr	—	—	—	—	4.8
					July–August					
1981	B	109.4	4.2	68.7	4.7	56.0	0.2	0.5	6.0	249.9
	C	90.0	9.0	9.4	8.7	6.2	0.4	0.7	11.4	135.7
1984	A	43.3	7.0	7.4	21.3	—	—	2.8	4.8	86.5
	B	28.3	32.1	18.1	4.3	—	38.7	0.1	0.4	122.2
	C	8.3	0.5	4.8	0.9	0.1	15.3	0.3	2.8	32.9
					September					
1982	B	762.2	14.2	56.7	102.2	20.2	1.0	2.0	53.6	1012.3
	C	293.0	0.4	0.4	18.3	93.9	—	—	0.4	406.4
1983	A	690.2	0.9	130.2	7.7	26.5	—	—	0.9	856.3
	B	112.5	0.1	—	6.8	—	—	—	8.7	128.1
	C	71.5	1.1	0.1	3.9	0.1	0.1	0.1	0.1	76.9
1984	A	81.5	25.4	107.9	104.0	0.7	—	0.3	6.2	326.0
	B	201.4	22.5	48.8	13.4	—	—	—	6.1	292.3
	C	34.5	1.0	—	0.5	2.6	0.1	—	—	38.7

salmon abundances and consumption rates in coastal waters to date. Such estimates are purely academic, of course, unless they are weighed against some reasonable estimates of the prey available to be consumed. Unfortunately, estimates of zooplankton biomass during the four years of interest are available only for 1981, which appears to be a lower than average year in both upwelling and productivity with only July showing above-average conditions (Brodeur and Pearcy 1992). The consumption estimates suggest that even during this year of lowered productivity, there was sufficient food available overall for the salmon. Much of this plankton biomass, however, was composed of taxonomic groups (e.g. chaetognaths) not normally consumed by coho or chinook salmon but utilized by other salmon and nonsalmonids (Brodeur et al. 1987).

Several other factors must be taken into consideration. The first is that these juvenile salmon are not the only pelagic predators placing a demand upon these zooplankton resources. Additional zooplanktivorous fishes present include other (although substantially less numerous) salmonid juveniles such as chum (*O. keta*), pink (*O. gorbuscha*), and sockeye salmon (*O. nerka*) and steelhead (*O. mykiss*) and cutthroat trout (*O. clarki*) (Pearcy 1984; Pearcy et al. 1990; Pearcy and Fisher 1990). Adult salmon of all species and numerous nonsalmonid predators (Brodeur and Pearcy 1986) are also found in pelagic waters and many of these fish prey on the same zooplankton taxa as juvenile coho and chinook salmon (Brodeur et al. 1987). For example, juvenile coho and chinook salmon together represent only 4.1, 18.0, 2.9, and 1.0% of the total abundance of pelagic nekton collected during May and June from 1981 through 1984, respectively (Brodeur and Pearcy 1992). Hence the feeding of these other nektonic species may seriously reduce the availability of food for juvenile salmon. During 1981, market squid (*Loligo opalescens*) and northern anchovy (*Engraulis mordax*) comprised 64.1 and 25.1%, respectively, of the total abundance of nekton caught. These predators consumed taxa (mainly chaetognaths and pteropods) that were not generally consumed by juvenile coho and chinook salmon and they showed little diet overlap ($\leq$13% in all cases) with juvenile salmon (Brodeur and Pearcy 1992). However, gelatinous macrozooplankton (Shenker 1984) and marine birds and mam-

TABLE 2. Daily consumption ($g \cdot km^{-2}$) of the major taxonomic groups by year–month–area subsets for average population sizes of juvenile coho salmon by the bioenergetic method. Dashes indicate that those prey were not found in that particular subset; tr = <0.1 $g \cdot km^{-2}$.

Year	Area	Fish	Decapods	Euphausiids	Amphipods	Pteropods	Insects	Copepods	Other	Total
					May					
1981	B	40.9	2.6	0.2	0.1	0.4	0.3	0.1	1.7	46.2
	C	54.2	15.2	3.5	1.2	21.8	—	0.3	12.1	108.3
1982	B	8.2	0.6	3.7	tr	—	tr	—	1.1	13.8
	C	119.0	17.1	14.9	3.0	1.5	—	0.7	7.4	163.6
1983	A	13.1	4.5	0.3	0.1	2.0	—	—	1.2	21.2
	B	31.9	2.4	3.5	1.5	tr	0.4	2.0	2.9	44.7
	C	35.4	1.0	3.2	0.5	tr	0.1	0.4	0.6	41.3
					June					
1981	B	187.3	14.0	9.8	1.2	3.1	—	1.2	7.3	224.0
	C	19.8	5.1	1.4	0.2	0.4	tr	tr	2.1	29.1
1982	B	124.4	9.7	31.4	8.7	0.5	2.7	0.5	17.3	195.3
	C	16.6	tr	1.9	tr	—	—	—	1.0	19.9
1983	A	37.5	9.4	0.2	0.3	—	0.2	0.1	1.9	49.6
	B	61.0	11.1	1.4	5.2	—	—	0.1	3.8	82.5
	C	28.9	1.7	1.5	0.9	—	tr	—	1.3	34.2
1984	A	44.4	7.5	3.4	1.5	—	—	tr	tr	57.0
	B	39.8	1.4	tr	—	—	—	—	tr	41.2
	C	4.8	0.7	4.3	tr	—	—	—	—	9.9
					July–August					
1981	B	111.9	4.3	70.5	4.7	70.9	0.3	0.5	7.9	271.0
	C	95.1	10.9	11.2	10.3	10.6	0.3	1.1	16.7	156.1
1984	A	60.1	10.0	6.1	21.7	—	—	2.0	3.0	102.7
	B	47.4	36.3	20.1	4.7	—	60.2	0.2	0.7	169.7
	C	19.7	1.8	10.7	0.8	0.1	30.2	0.6	5.5	69.3
					September					
1982	B	860.4	17.7	71.9	114.4	33.6	2.9	3.5	100.8	1205.4
	C	436.5	0.6	0.6	23.8	185.4	—	—	1.0	648.0
1983	A	652.6	1.0	123.3	7.3	39.4	—	—	1.0	824.6
	B	116.7	0.1	—	7.1	—	—	—	14.1	138.1
	C	71.0	1.2	0.6	3.7	0.1	0.1	0.1	0.6	77.4
1984	A	109.2	31.8	118.9	125.0	0.9	—	0.6	11.8	398.5
	B	212.1	32.8	56.4	15.1	—	—	—	9.2	325.2
	C	41.0	1.3	—	0.8	4.0	0.1	—	—	47.2

mals may also utilize many of the same resources so that much higher daily prey losses overall may be occurring than those estimated in this study.

Measures of zooplankton standing stock, however, do not reflect total production, as biomass is augmented daily by growth and new recruitment. Although the zooplankton sampling was not of sufficient intensity to estimate growth and recruitment, we were able to find values in the literature for similar species in each taxonomic group from which to make estimates for our study. Production rates are generally expressed per unit biomass as production to biomass ratios (P/B) or their inverses (B/P = turnover times). Typical turnover times for zooplankton in upwelling ecosystems are between 5 and 10 d (e.g. Shannon and Field 1985). Fish generally live much longer than zooplankton and have considerably lower production per unit biomass. Generally, average annual P/B ratios are on the order of 6.5 for copepods and other small zooplankton, 6.0 for euphausiids, and 0.5–2.0 for small juvenile fishes and 0.3 for adult pelagic fishes (Mauchline 1977; Mills and Fournier 1979; Hopkins et al. 1989). These values tend to be substantially higher during the summer months when much of the annual growth and recruitment occurs (Walters et al. 1978). Using these figures, the total production is approximately 5–6 times the standing stocks listed in Table 5 with the exception of fishes which have production values at most slightly exceeding the available biomass.

Finally, all prey available to plankton or any other sampling gear may not be equally available to a foraging salmon. Ney (1990) discussed three components of availability which may bias any field estimates of prey resources. The first is a morphological limitation that sets limits on the maximum and minimum prey sizes that are capable of being consumed by a predator of a particular size. The size of available prey may increase or decrease within a season but the size of the salmon predators generally increases throughout the summer (Brodeur 1991). Restricting the zooplankton size fraction we considered to those organisms >5 mm minimizes the limitation at the smaller prey sizes but many larger individuals, particularly fishes and cephalopods, can easily evade plankton nets (see below).

Another limitation to availability is that associated with the distribution of predators and prey. Most of the invertebrate zooplankton species and many of the fish larvae and juveniles are known to undertake extensive vertical migrations over the diel cycle. Superimposed on these movement patterns may be diel

TABLE 3. Daily consumption ($g \cdot km^{-2}$) of the major taxonomic groups by year–month–area subsets for average population sizes of juvenile chinook salmon by the field method. Dashes indicate that those prey were not found in that particular subset; tr = $<0.1\ g \cdot km^{-2}$.

Year	Area	Fish	Decapods	Euphausiids	Amphipods	Pteropods	Insects	Copepods	Other	Total
					May					
1981	B	6.8	tr	0.1	0.1	tr	—	tr	0.1	7.1
	C	3.6	0.1	0.8	0.1	tr	2.0	tr	—	6.6
1982	B	21.3	1.0	0.4	0.2	0.1	0.2	—	tr	23.2
	C	14.1	—	1.8	tr	tr	—	—	tr	16.0
1983	A	33.3	5.0	0.8	0.1	0.1	tr	tr	0.6	39.9
	B	27.9	2.1	0.2	tr	—	—	tr	0.9	31.3
	C	3.6	—	—	—	—	—	—	—	3.6
					June					
1981	B	9.3	1.4	0.1	tr	—	—	—	tr	10.9
	C	3.7	0.2	0.1	0.1	tr	1.5	tr	tr	5.7
1982	B	37.1	1.1	0.5	0.2	tr	—	tr	0.6	39.6
	C									1.1
1983	A	9.3	2.6	—	0.3	—	—	0.2	—	12.4
	B	11.9	0.6	tr	tr	tr	1.2	—	0.8	14.5
	C									15.8
1984	A	13.8	0.9	tr	tr	—	tr	0.7	tr	15.6
	B	17.2	1.2	0.4	—	—	0.8	tr	0.1	19.8
	C									2.4
					July–August					
1981	B	51.2	1.2	4.4	0.7	1.6	—	0.4	2.0	61.5
	C									5.9
1984	A	21.4	2.3	1.4	1.1	—	—	0.7	0.1	27.0
	B									13.9
	C									7.3
					September					
1982	B									8.8
	C									91.8
1983	A									33.9
	B	217.3	2.5	—	5.2	—	—	0.2	0.9	226.1
	C	524.3	2.1	0.5	1.6	—	2.7	—	0.5	531.7
1984	A	3.3	2.2	24.6	0.3	—	0.6	—	2.2	33.2
	B	35.7	1.6	0.4	0.4	—	—	—	tr	38.2
	C	78.6	1.3	0.2	2.0	0.2	—	—	0.6	82.9

differences in the vertical distribution of juvenile salmon (Pearcy and Fisher 1988) that may or may not coincide with that of their prey. Although the zooplankton tows used in this study were made throughout the water column, zooplankton generally occur in patches at various depths which may bias estimates of availability (Peterson et al. 1982). Prey patchiness can also occur along horizontal gradients at various spatial and temporal scales (Healey 1991).

The final category Ney (1990) mentioned is that of behavioral availability. Juvenile salmon, particularly those raised in hatcheries, may develop an inflexible search image that limits which animals they recognize as prey. Substantial seasonal or spatial differences in prey composition may require some adjustment on the part of the salmon in order to capture novel prey items (cf. Paszkowski and Olla 1985). Many prey species have also developed complex antipredation behaviors in order to survive. Among these may be transparency (chaetognaths and other gelatinous zooplankton), countershading (fishes and squid), evasive responses (many crustacean zooplankton), and schooling behaviors (fishes).

With the help of the above analysis, we can speculate on what the predation impact of salmon might be during a year such as the El Niño year of 1983 when productivity was even more reduced than in 1981. Although we do not have similar estimates of zooplankton standing stocks during 1983, other studies (Sefton et al. 1984; McGowan 1985; Miller et al. 1985) suggest that overall abundance and biomass were much reduced and perhaps only 10–50% of normal levels. Multiplying the midpoint (0.30) of this range by the 1981 zooplankton biomass and comparing the resulting available biomass with the 1983 consumption estimates by the field method for May and June, we estimate that between 0.09 and 0.16% of the total plankton biomass was consumed daily at the estimated average salmon abundance and between 0.12 and 0.21% was consumed daily at the maximum abundance levels (Table 7). Consumption estimates of euphausiids were quite low ($\approx 0.01\% \cdot d^{-1}$) but these prey were also a major food (≈90% of the diet by weight) of large schools of Pacific (*Scomber japonicus*) and jack mackerel (*Trachurus symmetricus*) that migrated into this coastal ecosystem during 1983 (Brodeur and Pearcy 1992). In contrast,

TABLE 4. Daily consumption (g·km $^{-2}$) of the major taxonomic groups by year–month–area subsets for average population sizes of juvenile chinook salmon by the bioenergetic method. Dashes indicate that those prey were not found in that particular subset; tr = <0.1 g·km $^{-2}$.

Year	Area	Fish	Decapods	Euphausiids	Amphipods	Pteropods	Insects	Copepods	Other	Total
					May					
1981	B	6.3	0.1	0.1	0.1	tr	—	tr	0.1	6.8
	C	3.4	0.1	0.7	0.1	tr	1.9	tr	—	6.3
1982	B	30.6	1.4	0.7	0.4	0.1	0.1	—	tr	33.3
	C	20.3	—	2.6	0.1	tr	—	—	tr	23.1
1983	A	32.7	3.6	0.6	0.1	0.1	tr	tr	0.6	37.7
	B	41.8	3.2	0.4	0.2	—	—	tr	1.4	47.1
	C	7.4	—	—	—	—	—	—	—	7.4
					June					
1981	B	13.2	1.6	0.3	tr	—	—	—	tr	15.2
	C	5.0	0.6	0.1	0.1	tr	2.1	tr	tr	7.9
1982	B	53.6	2.2	1.0	0.4	tr	—	tr	0.8	58.1
	C									2.0
1983	A	8.4	2.2	—	0.3	—	—	tr	—	11.0
	B	10.4	0.5	tr	tr	tr	1.0	—	0.7	12.7
	C									11.0
1984	A	17.5	1.1	0.2	0.1	—	tr	0.8	tr	19.8
	B	18.3	1.5	0.3	—	—	0.9	tr	0.1	21.1
	C									6.0
					July–August					
1981	B	50.0	1.2	4.3	0.7	1.6	—	0.2	2.0	60.0
	C									7.0
1984	A	27.5	3.3	1.4	1.4	—	—	0.9	0.1	34.6
	B									15.0
	C									9.0
					September					
1982	B									9.0
	C									125.0
1983	A									38.0
	B	176.3	2.6	—	4.2	—	—	0.2	0.7	184.0
	C	462.7	1.7	0.5	0.9	—	2.3	—	0.4	468.5
1984	A	3.7	2.4	26.8	0.3	—	0.6	—	2.4	36.2
	B	30.7	2.5	0.4	0.3	—	—	—	tr	33.9
	C	73.2	1.1	0.2	1.7	0.2	—	—	0.5	76.9

TABLE 5. Summary of available zooplankton based on bongo net collections during each time period. Data are mean wet weights (g) per 10 m^2 of sea surface with standard errors of the means in parentheses. Number of collections during each cruise are listed below each month.

Time period	Fish	Euphausiids	Decapods	Chaetognaths	Amphipods	Copepods	Pteropods	Other	Total
May (n = 28)	0.22	17.82	0.31	1.45	0.05	0.04	0.35	0.21	20.45
	(0.06)	(9.97)	(0.09)	(0.55)	(0.01)	(0.02)	(0.18)	(0.08)	
June (n = 29)	0.26	11.35	0.48	3.71	0.18	0.09	0.08	0.15	16.30
	(0.07)	(5.47)	(0.09)	(1.40)	(0.04)	(0.03)	(0.03)	(0.05)	
July–August (n = 29)	0.21	20.21	0.24	10.11	0.10	0.20	0.02	0.41	31.50
	(0.09)	(8.47)	(0.06)	(2.36)	(0.02)	(0.08)	(0.02)	(0.15)	

estimated fish consumption was extremely high (6.5–10.7%·d^{-1}) and assuming average P/B ratios as low as 0.5–2.0, the available fish resources could easily be exhausted by juvenile coho and chinook salmon as well as other predators utilizing fish as prey.

The estimates of fish biomass available, in particular, appear to be very low and considering the fact that fishes are the main food of these salmon species, fish consumption at the rates estimated in this study would quickly deplete the available resources. It should be emphasized here that the bongo gear we used is not an adequate sampler for larger fishes. Late larval and early juvenile fishes are able to evade the mouth opening of most plankton gear and require much larger sampling devices (Methot 1986; Shenker 1988). Therefore, the prey fish abundances estimated from the plankton sampling may be suitable only for the early summer months (May and June), when juvenile coho and chinook are consuming mainly fish larvae and juveniles, but they are probably poor estimates of food avail-

TABLE 6. Comparison of combined coho and chinook salmon daily consumption of major prey taxa and average total biomass for areas B and C during 1981 with estimates of available prey from plankton tows. All weights are standardized to 1 km^2 of sea surface area. Consumption was estimated at average and at maximum population sizes for both salmon species.

Time period	Food category	Consumed daily by salmon (g)		Available food (g)	Percent consumed per day	
		Average	Maximum		Average	Maximum
May	Fish	51.9	60.2	2 200	2.36	2.73
	Euphausiids	2.2	2.6	178 200	0.00	0.00
	Decapods	8.8	10.2	3 100	0.28	0.33
	Total	79.7	92.5	204 500	0.04	0.05
June	Fish	103.3	123.8	2 600	3.97	4.76
	Euphausiids	11.5	13.7	113 500	0.01	0.01
	Decapods	9.9	11.8	4 800	0.21	0.25
	Total	131.2	157.2	163 000	0.08	0.10
July–August	Fish	127.8	140.0	2 100	6.09	6.67
	Euphausiids	41.5	45.4	202 100	0.02	0.02
	Pteropods	32.1	35.1	200	16.03	17.55
	Total	226.5	246.9	315 000	0.07	0.08

TABLE 7. Comparison of combined coho and chinook salmon daily consumption of major prey taxa and average total biomass for areas B and C during 1983 with estimates of available prey calculated from 1981 data. All weights are standardized to 1 km^2 of sea surface area. Consumption was estimated at average and at maximum population sizes for both salmon species.

Time period	Food category	Consumed daily by salmon (g)		Available food (g)	Percent consumed per day	
		Average	Maximum		Average	Maximum
May	Fish	43.2	56.3	660	6.55	8.52
	Euphausiids	3.2	4.1	53 460	0.01	0.01
	Decapods	3.2	4.1	930	0.34	0.44
	Total	54.3	70.7	61 350	0.09	0.12
June	Fish	61.7	83.8	780	7.90	10.75
	Euphausiids	1.1	1.5	34 050	0.00	0.00
	Decapods	8.0	10.9	1 440	0.56	0.76
	Total	76.9	104.5	48 900	0.16	0.21

able for the larger juvenile salmon in late summer or early fall when their diet consists mainly of larger juvenile fishes (Brodeur and Pearcy 1990).

There are several reasons why exact agreement in total or prey-specific food consumption between the direct and bioenergetic methods would not be expected. The direct method provides merely a "snapshot" of food consumption at three different time periods over the entire summer and lacks information on what consumption takes place between sampling intervals. The bioenergetic model integrates consumption between these sampling periods but may be sensitive as to which value is chosen to represent the monthly consumption, since consumption increases exponentially with size of fish during any time period. We chose the consumption level at the midpoint of the month in the present analysis and thus a cruise occurring at the beginning or end of the month would be expected to generate much different consumption between the field and bioenergetic approaches. Moreover, the bioenergetic model would be expected to be sensitive to errors in growth measurements attributable to size-selective mortality (Cochran and Knutsen 1988). Evidence for size-selective marine mortality in the population we examined is equivocal (Fisher and Pearcy 1988) and the magnitude of the error resulting from this bias was found to be negligible (<3.5%) in the one instance it has been examined (Cochran and Knutsen 1988).

Although both methods of estimating food consumption incorporate several assumptions and suffer from potential biases inherent in the methodology, the close agreement of the two methods increases confidence in the overall results. Similarities between bioenergetic and in situ estimates of consumption in other studies (Rice and Cochran 1984; Beauchamp et al. 1989; Ruggerone and Rogers 1992) provide evidence of the utility of bioenergetic modelling as a corroboration of the field estimates. It must be kept in mind, however, that several independent methods of estimating food consumption may yield different results (Olson and Boggs 1986) in which case it may be difficult to determine which most closely approximates the consumption levels occurring in the natural environment.

Our estimates of total daily consumption as a percentage of the total available food (≈0.1–0.2%) consumed by coho and chinook salmon combined are similar to cruder estimates (≈0.2–0.4%) made earlier based on prey numbers consumed and estimated prey abundance (Peterson et al. 1982). The latter estimate is probably too high, since it was based on the premise that salmon fill their stomachs three times a day, which does not appear to be correct (Brodeur and Pearcy 1987). Our results

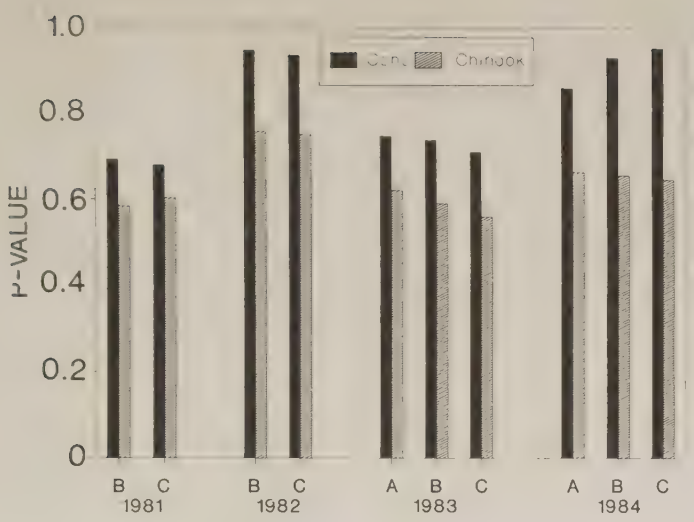


FIG. 10. Proportion of the maximum ration expected for each year–area subset generated by the bioenergetic model for juvenile coho and chinook salmon.

concur with the earlier estimates (Peterson et al. 1982; Walters et al. 1978) that these salmon juveniles generally do not appear to be food limited in coastal waters during most normal years but they show that there is a possibility that food limitation can occur during some low-productivity years (e.g. 1983) or in some poor-productivity areas.

This food limitation would more likely manifest itself in poor growth or condition of individual fish (Fisher and Pearcy 1988), rather than mass starvation of a cohort, making these fish more susceptible to size-selective predators (Pearcy 1988). One output parameter (P) generated by the bioenergetic model represents the proportions of the theoretical maximum possible for a particular species at the observed weights and temperatures and is a useful index of the degree of food limitation in the population (Rice and Cochran 1984). Based on the model results (Fig. 10), neither species appears to obtain the maximum possible ration that it is theoretically capable of consuming. Although ideal feeding conditions are seldom met in nature whereby these theoretical maxima can be obtained, these deficiencies appear to be particularly acute for chinook salmon juveniles and for both species during warm or low-productivity years (1981 and 1983). Fisher and Pearcy (1988) found that these two years were the only ones among the four years examined here which showed evidence of size-selective mortality occurring for coho salmon early in the summer, despite a general lack of a relationship between early ocean growth and survival.

Although food limitation may be the proximate cause of low early marine survival in some years, the ultimate cause of death is likely to be predation on the slower growing or physically weakened individuals. Salmon smolts may be most vulnerable when they leave the estuary and enter the coastal environment, where they must quickly adapt to a new suite of prey resources as well as an entirely different predatory field. The most intense predation on slow-growing individuals is likely to occur during the first few weeks to a month at sea. Therefore, those fish that escape predation may acquire enough food to continue growing somewhat below their maximum potential.

Acknowledgements

This study was completed in partial fulfillment of the requirements for a Ph.D. degree at the University of Washington by the senior author. We thank J. Fisher for providing us with his unpublished growth data for juvenile chinook salmon and D. Beauchamp for assistance with the bioenergetic modelling. We also appreciate the helpful comments and suggestions provided by D. J. Stewart and M. C. Healey. This study was funded by a grant from the National Coastal Resources and Development Institute.

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Assessment of the Impact of Heavy Metals on Benthic Communities at the Clinch River (Virginia): Evaluation of an Index of Community Sensitivity

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Clements, W. H., D. S. Cherry, and J. H. Van Hassel. 1992. Assessment of the impact of heavy metals on benthic communities at the Clinch River (Virginia): evaluation of an index of community sensitivity. Can. J. Fish. Aquat. Sci. 49: 1686–1694.

Benthic communities (sampled once annually from 1986 to 1989) at stations impacted by copper and zinc were characterized by reduced species richness, reduced abundance, and a shift in community composition from sensitive to tolerant taxa. Benthic community structure varied annually due to fluctuations in stream discharge and improvements in water quality. Sensitivity of 13 dominant taxa to Cu was measured in outdoor experimental streams by exposing organisms to Cu (25 μg/L) for 10 d. Sensitivities (s_i), defined as proportioned reduction in abundance in treated streams relative to controls, ranged from 1.00 for several species of Ephemeroptera that were completely eliminated to −0.14 for taxa that increased in treated streams (Chironomidae: Orthocladiini). An index of community sensitivity (ICS) based on s_i and relative abundance of dominant taxa (p_i) was developed for the Clinch River. The ICS was highly sensitive to heavy metals and useful for distinguishing reference and impacted stations. There was good agreement between the ICS and other community-level approaches in identifying impacted stations. We suggest that specific ICS values, based on s_i estimates of dominant taxa within a region, may be employed to predict the degree of metal impact in other streams within that region.

Les communautés benthiques (échantillonnées une fois par an de 1986 à 1989) des stations touchées par le cuivre et le zinc ont été caractérisées par une richesse en espèces réduite, une abondance réduite et une modification de la composition des communautés, passant des taxons vulnérables aux taxons tolérants. La structure des communautés benthiques variait sur une base annuelle en raison des fluctuations du débit des cours d'eau et des améliorations de la qualité des eaux. La sensibilité de 13 taxons dominants vis-à-vis du Cu a été mesurée dans des cours d'eau expérimentaux extérieurs en exposant les organismes à 25 μg Cu/L pendant 10 jours. Les sensibilités (s_i), définies comme étant la réduction proportionnelle de l'abondance dans les cours d'eau traités par rapport aux témoins, variaient de 1,00 pour plusieurs espèces d'éphéméroptères qui ont été complètement éliminées à −0,14 pour les taxons dont l'abondance a augmenté dans les cours d'eau traités (Chironomidae : orthocladiini). Un indice de la sensibilité des communautés (ISC) basé sur s_i et sur l'abondance relative des taxons dominants (p_i) a été développé pour la rivière Clinch. L'ISC était extrêmement sensible aux métaux lourds et s'est avéré utile pour distinguer les stations de référence et les stations touchées. On a trouvé une bonne corrélation entre l'ISC et d'autres approches axées sur les communautés pour l'identification des stations touchées. Il serait donc possible d'utiliser certaines valeurs spécifiques de l'ISC, basées sur les estimations de la s_i des taxons dominants au sein d'une région, pour prédire le degré de l'impact des métaux dans les autres cours d'eau de cette région.

Received September 6, 1991
Accepted February 14, 1992
(JB210)

Reçu le 6 septembre 1991
Accepté le 14 février 1992

Analysis of the distribution and abundance of benthic invertebrates is routinely employed in stream biomonitoring studies to examine the impact of heavy metals. Benthic invertebrates are good indicators of water quality because of their wide variation in sensitivity to contaminants and relative immobility (Wiederholm 1984; Voshell et al. 1989). Metal-impacted benthic communities are generally characterized by reduced abundance, lower species diversity, and shifts in community composition from sensitive to tolerant taxa (Winner et al. 1980; La Point et al. 1984; Waterhouse and Farrell 1985; Wiederholm 1984; Chadwick et al 1986; Clements 1991a).

Effects of heavy metals on benthic communities are often confounded by natural variation in community structure and function. Variation in abundance, species diversity, and community composition along longitudinal stream gradients is well documented (Vannote et al. 1980). Species richness, an index commonly employed in stream biomonitoring studies, generally increases from headwaters to midorder streams (Stout and Vandermeer 1975; Minshall et al. 1985). Consequently, it is often difficult to separate effects due to perturbations from those due to natural longitudinal changes in community structure.

These limitations demonstrate the need to develop objective methods that are less sensitive to variation among upstream and downstream sites for evaluating the impact of heavy metals. Hilsenhoff's biotic index (Hilsenhoff 1987) is a recent approach for assessing impact of organic enrichment on streams. It integrates field data on benthic community composition with estimates of relative sensitivity of dominant taxa. Scores are

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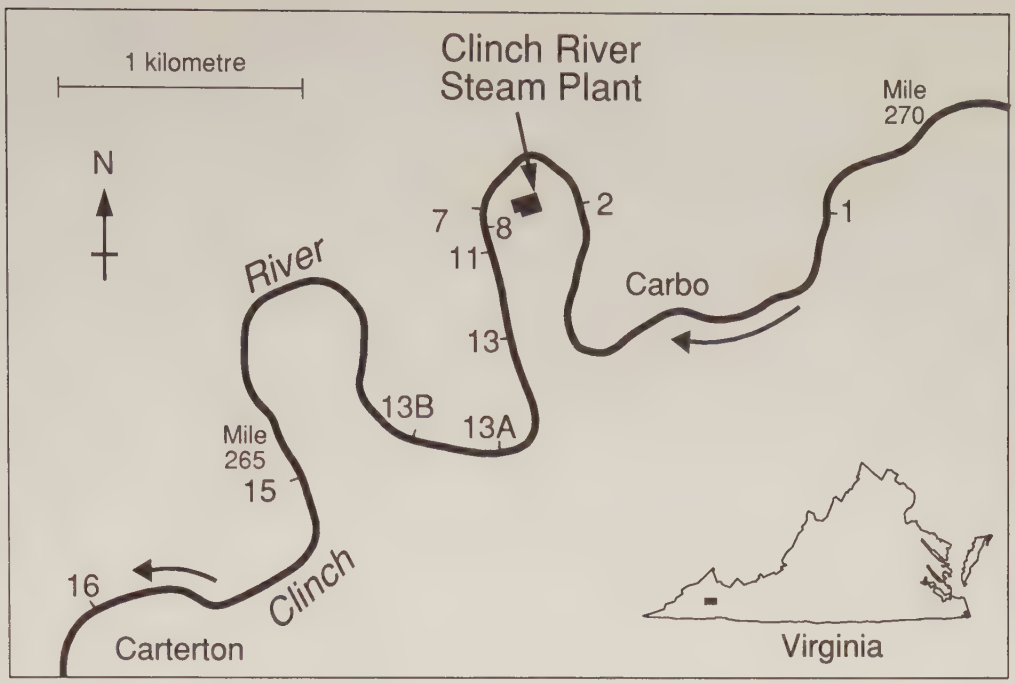

FIG. 1. Sampling locations at the Clinch River, Virginia.

assigned to sampling locations based on the relative abundance of sensitive and tolerant groups. Although the biotic index is useful for assessing impacts of organic enrichment, the applicability of this index to other classes of contaminants (e.g. heavy metals, acidification, and pesticides) is uncertain. Furthermore, while reasonably accurate estimates of relative abundance may be obtained by field sampling, species-specific sensitivity of benthic invertebrates is difficult to estimate.

Despite evidence of considerable variation in responses of stream invertebrates to heavy metals, there have been few attempts to obtain metal-tolerance estimates specific for these organisms. Several investigators have noted a general decrease in tolerance to heavy metals from chironomids to caddisflies to stoneflies to mayflies (Sprague et al. 1965; Winner et al. 1980; Clements et al. 1988; Clements 1991a). However, within each of these broad taxonomic groups, there is considerable variation in effects of these contaminants.

This paper examines annual variation in responses of benthic communities to heavy metals. We describe an experimental approach for estimating sensitivity of benthic invertebrates to heavy metals in outdoor stream mesocosms. Estimates of sensitivity are integrated with estimates of relative field abundance to obtain an index of community sensitivity for the Clinch River, a system impacted by copper and zinc. The usefulness of this index for describing spatial (among stations) and temporal (among years) variation is compared with that of several other community level approaches.

Materials and Methods

Sampling Benthic Communities

Benthic communities were sampled once annually (six to eight stations per year) between 1986 and 1989 at the Clinch River (Russell Co., Virginia), a southwest Virginia stream receiving Cu and Zn from a coal-fired power plant (Fig. 1). Samples were collected during late summer under low-flow conditions each year (14 August to 17 September) using substrate-filled trays. Six substrates (10 × 10 × 6 cm trays filled with pebble and small cobble) were placed at each station. Details of the Clinch River system and the substrate-filled trays have been reported previously (Clements et al. 1988, 1989c). Stations 1, 2, and 7 were located upstream of the Clinch River Plant (CRP) and served as reference sites. Station 8 was located approximately 50 m downstream of a cooling tower effluent containing elevated levels of Cu and Zn. The remaining six stations were located downstream of the CRP. Stations 7, 8, and 11 were sampled only in 1986, while stations 13A and 13B were added in 1987. Samples were not collected from station 1 in 1987 and station 16 in 1989 due to loss of trays. Generally, three to six trays were removed after 30 d of colonization. A 500-μm-mesh net was placed directly downstream to prevent the loss of organisms. Contents of each tray were washed through a 500-μm-mesh sieve in the field and organisms retained were preserved in 10% formalin. In the laboratory, all organisms except chironomids were identified to species or genus. Chironomids were identified to tribe.

Temperature, pH, conductivity, dissolved oxygen, hardness, and alkalinity were measured weekly at reference station 2. Differences in these water quality parameters between stations were minimal and have been reported previously (Clements et al. 1988). Stream discharge data were obtained from the nearest gauging station which was located in Cleveland, Virginia, approximately 6 km upstream from the plant. Estimates of effluent volume (cubic metres) were obtained from the CRP. Concentrations of Cu and Zn (total recoverable) in the effluent, at reference station 2, and at station 8 were measured weekly using atomic absorption spectrophotometry.

Changes in water quality at the Clinch River occurred between 1986 and 1989 (Table 1). Alkalinity, stream discharge, and effluent volume varied significantly among years. Stream discharge was lowest in 1987 and 1988 and was below the 20-yr average for the Clinch River during this period. Due to increased precipitation, stream discharge was significantly greater in 1989 than in other years.

TABLE 1. Water quality at the Clinch River, 1986–89. Data are means (±SD) of weekly samples (n = 5) from August to September. Data for temperature, pH, conductivity, hardness, alkalinity, and current velocity were collected from reference station 2. Levels of Cu and Zn (μg/L) were measured in the effluent, at station 2, and at station 8. Metal concentrations at station 2 are given as ranges, since most values measured were below detection (bd). Stream discharge was measured at a gauging station located upstream from the Clinch River Plant. One-way ANOVA was employed to test for differences among years. Means followed by the same letter were not significantly different based on Ryan's Q multiple range test. $*p < 0.05$; $**p < 0.001$; $***p < 0.0001$; ns = not significant.

	1986	1987	1988	1989	F-value
Temperature (°C)	22.6 (±1.9)	25.1 (±2.0)	23.4 (±4.5)	22.8 (±1.3)	0.84 ns
pH	8.46 (±0.11)	8.42 (±0.16)	8.14 (±0.18)	8.16 (±0.33)	3.05 ns
Conductivity (μmho)	302.2 (±19.2)	313.6 (±6.3)	325.8 (±25.6)	316.0 (±3.9)	1.74 ns
Dissolved oxygen (mg/L)	8.0 (±0.6)	7.3 (±0.3)	8.1 (±0.9)	8.5 (±0.8)	2.22 ns
Hardness (mg/L)	155.8 (±12.1)	153.8 (±11.1)	147.4 (±7.3)	155.6 (±5.6)	0.88 ns
Alkalinity (mg/L)	156.0 a (±20.3)	137.0 ab (±8.6)	126.6 b (±11.4)	132.0 b (±10.0)	4.57*
Discharge (m^3/s)	4.40 b (±1.47)	2.42 b (±0.55)	2.68 b (±1.77)	9.71 a (±3.58)	12.46**
Effluent volume (m^3/s)	0.05 b (±0.01)	0.07 b (±0.02)	0.12 a (±0.02)	0.06 b (±0.01)	11.28**
Effluent [Cu]	353.0 (±164.0)	480.0 (±182.0)	323.4 (±64.0)	260.4 (±210.0)	1.57 ns
Effluent [Zn]	204.6 a (±109.2)	173.6 ab (±102.1)	36.8 c (±14.0)	55.8 bc (±28.2)	5.86*
Station 2 [Cu]	bd −17.0	bd	bd −2.0	bd −15.0	
Station 2 [Zn]	bd −22.0	bd −30.0	bd	bd −27.0	
Station 8 [Cu]	104.8 ab (±42.3)	126.6 a (±42.8)	89.0 ab (±36.4)	52.2 b (±29.1)	3.66*
Station 8 [Zn]	81.2 a (±16.7)	52.2 ab (±44.0)	16.8 b (±8.9)	39.4 ab (±50.0)	3.74*

Concentrations of Cu and Zn at reference station 2 were frequently below detection, and therefore, mean values were not calculated. Concentrations of metals in the effluent and at station 8 varied among years as a result of changes in stream discharge and improvements in water quality. In late 1987, the CRP initiated a program that effectively reduced effluent metal concentrations in subsequent years. Effluent concentrations of Zn were approximately 75% lower in 1988 and 1989 compared with previous years. Although effluent concentrations of Cu were 30% lower after 1987, this difference was not significant owing to large variation among weeks. Concentrations of Cu and Zn measured at station 8 also varied significantly among years. In-stream levels of Cu remained elevated in 1988, possibly due to reduced stream discharge and less dilution.

Index of Community Sensitivity

Relative sensitivity of benthic invertebrates to heavy metals was estimated during August 1987 in outdoor stream mesocosms located at the CRP. Details of the stream mesocosms and experimental approach have been reported previously (Clements et al. 1988, 1989b). Substrate-filled trays were colonized at reference station 2. After 30 d, 36 trays were removed from the stream, placed in 7-L insulated containers (four trays per container), and transferred to each of nine outdoor streams (95 × 71 × 14 cm). The experimental streams received diluent water directly from the Clinch River, upstream from the cooling tower effluent. After 2 d of acclimation, the nine streams were randomly assigned to three Cu treatments: control (ambient) and 12 and 25 μg Cu/L. Two trays were removed from each stream after 4 and 10 d of exposure to Cu. Trays were collected and processed as described above.

Sensitivity (s_i) of the 13 dominant taxa to Cu was determined by measuring the reduction in abundance (relative to control streams) in the 25 μg Cu/L treatment after 10 d of exposure. These data were chosen to estimate sensitivity, since they showed the greatest separation among taxa (Clements et al. 1989b). Relative abundance of dominant taxa (p_i) was measured at upstream and downstream sites at the Clinch River. The index of community sensitivity (ICS) is given as

$$\text{ICS} = \Sigma s_i p_i$$

where s_i = proportion reduction in abundance of the ith species in treated streams relative to controls and p_i = proportion abundance of the ith species in Clinch River field samples. Low ICS values are expected at stations dominated by metal-tolerant organisms whereas high values are expected at stations dominated by metal-sensitive taxa. Note that negative ICS values are possible and are expected at stations dominated by organisms that increased in treated streams relative to controls.

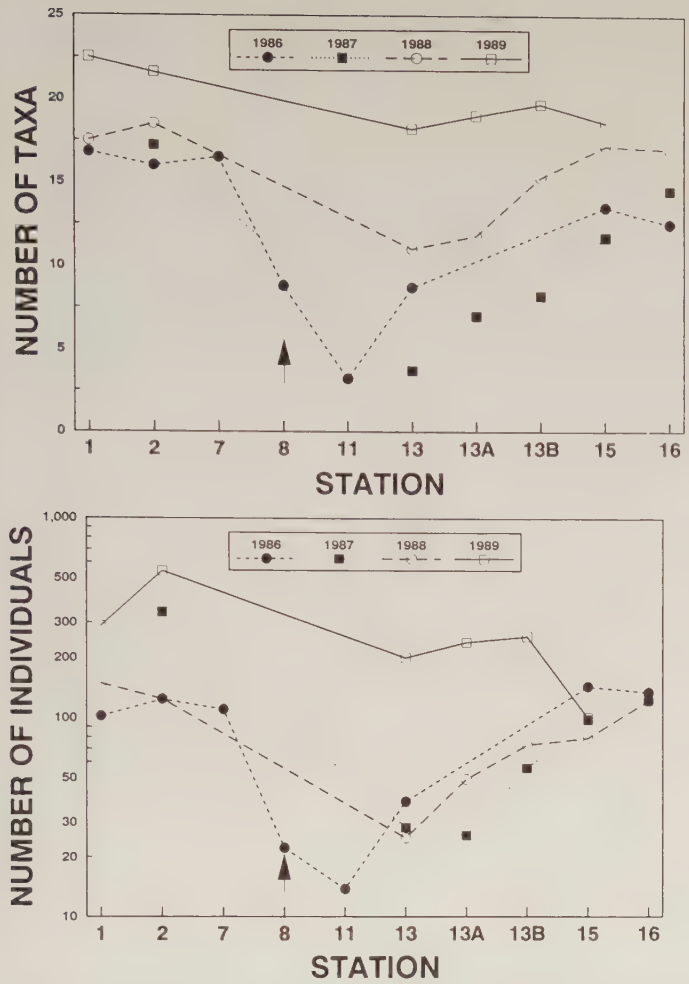

FIG. 2. Mean number of taxa and number of individuals at Clinch River stations between 1986 and 1989. Arrows indicate the location of cooling tower effluents.

Results

Effects of Metals on Number of Taxa, Number of Individuals, and Community Composition

Effects of CRP effluents on the number of taxa and individuals varied among stations and among years (Fig. 2). Effects of metals on the number of taxa and individuals were greatest between 1986 and 1988. In particular, during low-flow conditions in 1987 the mean number of taxa per tray was reduced from 17.2 ($\pm$2.4) at reference station 2 to 3.7 ($\pm$0.8) at station 13. Total abundance in 1987 at station 13 was reduced by >90% compared with reference station 2. Effects of CRP effluents on number of taxa were less severe in 1988 and 1989 following reduction of metal concentrations. Similarly, variation among stations in number of individuals was reduced in 1989.

Ephemeroptera (mayflies), Tanytarsini chironomids, Hydropsychidae (caddisflies), and Orthocladiini chironomids numerically dominated samples collected from all stations at the Clinch River and generally accounted for >80% of total individuals (Fig. 3). Benthic community composition was highly sensitive to heavy metals and varied among locations and years. Reference stations each year were generally dominated by several species of mayflies (*Baetis brunneicolor, Isonychia bicolor, Stenonema modestum, Tricorythodes* sp., and *Caenis* sp.) and Tanytarsini chironomids whereas impacted sites were dominated by Orthocladiini chironomids and net-spinning caddisflies (*Hydropsyche bifida* and *Cheumatopsyche* sp.). In 1986 and 1987, Orthocladiini chironomids at downstream station 13 comprised 51 and 82% of total individuals, respectively. The severe impact of metals on benthic community composition in 1987 was demonstrated by reduced abundance of Ephemeroptera, which comprised less than 5% of total individuals at the farthest downstream station.

Benthic community composition was less variable among stations in 1988 and 1989 following improvements in water quality. With the exception of station 13A in 1988, Orthocladiini chironomids comprised less than 20% of total abundance at any downstream station. Percent composition of mayflies and Tanytarsini chironomids was greater at downstream stations in 1988 and 1989 than in 1987.

Multivariate Analysis of Community Composition

Canonical discriminant analysis (Dillon and Goldstein 1984) was employed to analyze overlap and separation of Clinch River sampling stations each year. This multivariate technique separates groups (= stations) based on linear combinations of variables. Variables included in these analyses were the eight dominant taxa (*Isonychia bicolor*, Baetidae, *Stenonema* sp., *Tricorythodes* sp., *Caenis* sp., Hydropsychidae, Orthocladiini, and Tanytarsini) collected from the Clinch River between 1986 and 1989.

Canonical variables one and two (CAN1 and CAN2) together accounted for 76–98% of the total variation among stations each year (Fig. 4). In 1986, stations located closest to the CRP (8, 11, and 13) were clearly separated from all other sites. Downstream stations 15 and 16 were grouped with reference stations 2 and 7. Mayflies (all species) and Tanytarsini chironomids were the most important variables separating stations along the CAN1 axis in 1986. The severe effect of metals on benthic community structure in 1987 was demonstrated by the degree of separation of reference station 2 from all downstream stations. Separation of stations along CAN1, which explained 84% of the total variance, resulted from reduced abundance of Baetidae, *Stenonema* sp., and Tanytarsini chironomids at downstream stations. Canonical discriminant analysis suggested reduced effects of heavy metals on benthic communities in 1988 compared with 1987. Only stations 13 and 13A were clearly separated from upstream reference stations. Separation among stations observed in 1989 was probably not a result of CRP effluents, since benthic communities at reference station 2 were similar to those at downstream station 13.

Index of Community Sensitivity

Benthic communities in outdoor experimental streams were numerically dominated by Ephemeroptera, Trichoptera, and Chironomidae. Thirteen dominant taxa comprised greater than 90% of the total individuals in these streams. Sensitivity values (s_i) were assigned to these 13 dominant taxa based on the proportion reduction in treated streams relative to controls. Sensitivity values ranged from 1.00 for species completely eliminated in treated streams to -0.14 for Orthocladiini (which increased by 14.5% in treated streams) (Table 2). In general, mayflies were the most sensitive organisms examined. The proportion reduction of mayflies in treated streams compared with controls ranged from 0.67 (*Tricorythodes* sp.) to 1.00 (*Isonychia bicolor* and *Pseudocloeon* sp.). Tanytarsini chironomids were also highly sensitive to Cu exposure and were reduced by 0.80 in treated streams. Sensitivity of net-spinning caddisflies

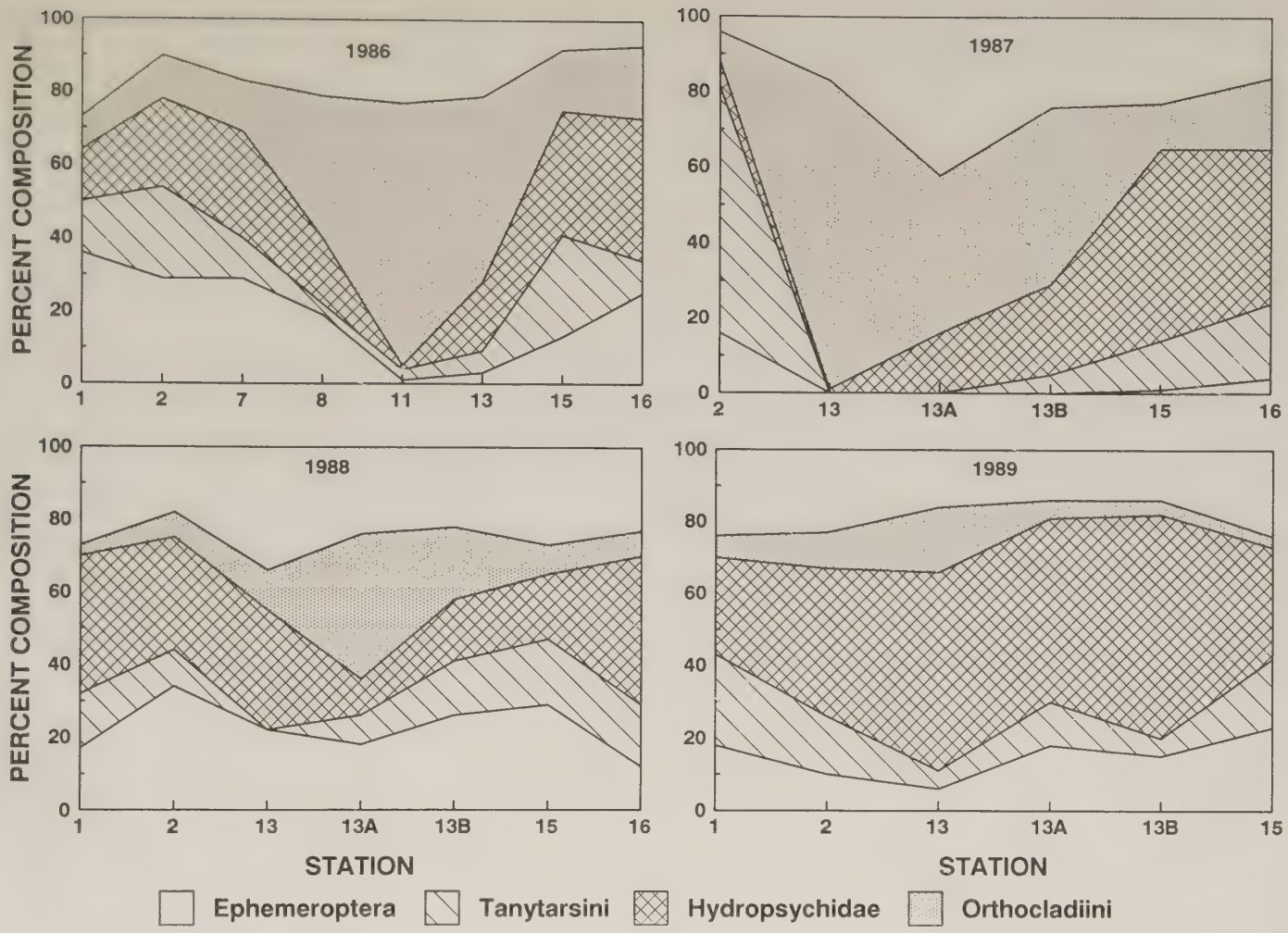

FIG. 3. Percent composition of dominant groups at Clinch River stations between 1986 and 1989.

(*Cheumatopsyche* sp. and *Hydropsyche bifida*) was intermediate between mayflies and Orthocladiini and ranged from 0.16 to 0.30.

ICS, derived from s_i and p_i values at the Clinch River (Table 2), was calculated for all stations (Fig. 5). ICS values at reference stations ranged from 0.30 to 0.66. The index was highly sensitive to heavy metals and reflected both spatial and temporal changes in benthic communities. As with the other indices examined, the effects of CRP effluents were greatest in 1986 and 1987. ICS values at stations 15 and 16 approached reference station values in 1986 but remained below reference station values in 1987. During this year when stream discharge was lowest, ICS values ranged from 0.66 at reference station 2 to −0.12 at impacted station 13. Severe reduction in ICS values at downstream stations in 1987 resulted from increased relative abundance of highly tolerant (Orthocladiini) and moderately tolerant (Hydropsychidae) organisms. Variation in ICS among stations was reduced in 1988 and 1989 compared with previous years. The lowest ICS value was measured at station 13A (0.17) in 1988 and negative values were not recorded in either year.

Comparison of Community-Level Approaches for Assessing Impacts of Heavy Metals

There was considerable variation between years in each of the community-level parameters measured at Clinch River reference stations (Fig. 2 and 5). In contrast, variation among reference stations within the same year was generally much less. Mean reference values were calculated each year for all upstream sites combined (except in 1987 when only one reference station was sampled). The number of individuals was the most variable, ranging from 112 per sample in 1986 to 403 per sample in 1989 (Table 3). Reference station values were compared with downstream sites using one-way ANOVA and Ryan's Q multiple range tests. Ryan's Q is a conservative test that has been recommended because of its rigorous control of experimentwise error rates (Day and Quinn 1989). Downstream stations were considered to be impacted if the number of taxa or individuals or ICS values were significantly less than the mean reference station values. There was generally good agreement among each of the community parameters in identifying impacted stations. Except for 1987, the set of stations defined as impacted based on reduced number of taxa or ICS values were identical. Similarly, stations defined as impacted based on the ICS in 1986, 1987, and 1988 were clearly separated from reference stations based on canonical discriminant analysis.

Discussion

Spatial and Temporal Variation in Benthic Communities

Benthic community structure at the Clinch River was highly sensitive to heavy metals and therefore useful for assessing the influence of CRP effluents. Differences among stations in number of taxa, number of individuals, percent composition of

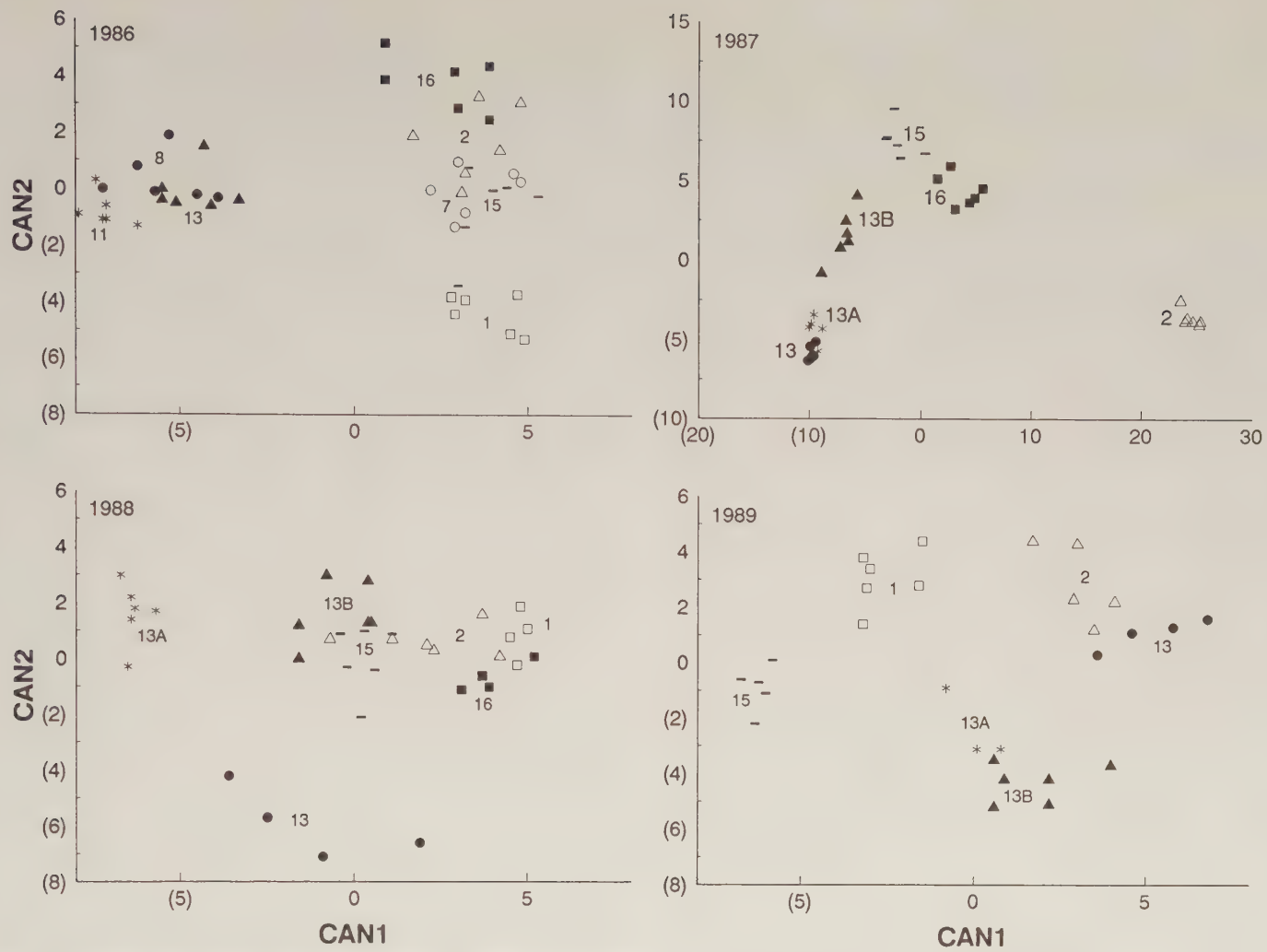

FIG. 4. Canonical discriminant analysis of benthic community composition at the Clinch River, 1986–89. Analyses were based on abundance of the eight dominant taxa (*Isonychia bicolor*, Baetidae, *Stenonema* sp., *Tricorythodes* sp., *Caenis* sp., Hydropsychidae, Orthocladiini, and Tanytarsini) collected at the Clinch River. Stations are indicated in each panel. Each point represents a single sample.

dominant taxa, and an index of community sensitivity were observed each year. Canonical discriminant analysis also showed distinct separation among reference and impacted sites in 1986, 1987, and 1988.

Annual variation in benthic community composition resulted from both improvements in water quality and variation in stream discharge (Table 1). Each of the community variables examined indicated that effects of CRP effluents were greatest during the period of low-flow conditions in 1987. We hypothesize that a combination of reduced stream discharge, higher water temperature, and high levels of Cu and Zn contributed to the greater effects observed in 1987. Results of two-way ANOVA showed highly significant station × year interaction for number of taxa ($F = 15.0$; $p < 0.001$), number of individuals ($F = 21.5$; $p < 0.001$), and ICS values ($F = 47.5$; $p < 0.001$). These results demonstrate that effects of metals on benthic community structure were dependent on year.

Despite improved water quality at the Clinch River in 1988, effects of metals on benthic community structure were still apparent. It is possible that differences observed between 1988 and 1989 resulted from insufficient time for this system to recover. Few studies have investigated recovery of metal-impacted streams following improvements in water quality (Chadwick et al. 1986; Leland et al. 1989). Alternatively, differences in effects of CRP effluents between 1988 and 1989 may be related to differences in stream discharge and effluent volume. Because of low stream discharge and high effluent volume in 1988, the percent effluent volume was much greater in 1988 (4.5%) than in 1989 (0.6%). Consequently, it is difficult to determine the degree of recovery of the Clinch River system. These results demonstrate the importance of assessing temporal variation in benthic community composition in stream biomonitoring studies.

Comparison of Community-Level Approaches for Assessing Impacts of Heavy Metals

Each of the approaches employed in this study was useful for separating reference and impacted sites at the Clinch River. Despite the generally good agreement among these approaches, there were important differences, particularly when effects of metals were less severe. In 1988, stations 13, 13A, 13B, and 15 were identified as impacted based on one-way ANOVA of the number of individuals. Each of the other approaches identified only stations 13 and 13A as impacted. In 1989 the number of individuals at station 15 was significantly less than reference values. Canonical discriminant analysis also showed that this station was distinct from reference sites. In contrast, station 13 was identified as impacted based on results of one-way ANOVA of the ICS and number of taxa. Differences among these approaches in identifying impacted stations illustrate the limi-

TABLE 2. Sensitivity (s_i) and proportion abundance (p_i) of dominant benthic invertebrates collected from the Clinch River. Sensitivity was defined as the proportion reduction in treated streams (25 μg Cu/L) compared with controls after 10 d of exposure. Proportion abundance data and examples of ICS calculations are shown for stations 2 and 13 in 1986.

Taxa	s_i	p_i	
		Station 2	Station 13
Ephemeroptera			
Isonychia bicolor	1.00	0.05	0.00
Pseudocloeon sp.	1.00	0.01	0.00
Baetis brunneicolor	0.99	0.07	0.02
Caenis sp.	0.95	0.02	0.00
Stenonema modestum	0.73	0.07	0.00
Tricorythodes sp.	0.67	0.05	0.00
Trichoptera			
Neureclipsis sp.	0.53	0.01	0.00
Cheumatopsyche sp.	0.30	0.18	0.16
Hydropsyche bifida	0.16	0.07	0.05
Diptera			
Tanytarsini	0.79	0.23	0.06
Chironomini	0.70	0.05	0.02
Tanypodini	0.30	0.01	0.01
Orthocladiini	−0.14	0.13	0.51
ICS = $\Sigma s_i p_i$		0.50	0.07

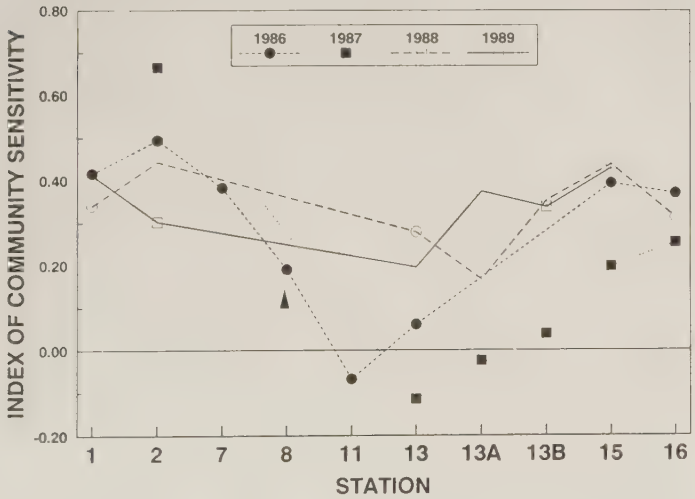

FIG. 5. ICS values at Clinch River stations between 1986 and 1989. The arrow indicates the location of cooling tower effluents.

tations of any single index. Therefore, we recommend that several indices be measured simultaneously to assess the effects of contaminants on benthic communities.

The use of multivariate statistics to analyze benthic community responses to contaminants in lotic systems has received only limited attention (Leland et al. 1986). Although multivariate procedures have been criticized and may not be appropriate for hypothesis testing (Rexstad et al. 1988), in biomonitoring studies they provide additional evidence for identifying impacted stations. In the present study, canonical discriminant analysis detected both spatial and annual variation in community composition at the Clinch River. These results were generally in good agreement with results of univariate analyses. We conclude that canonical discriminant analysis was a useful approach for assessing impacts of Clinch River effluents on benthic community composition.

Index of Community Sensitivity

Differences among groups of aquatic insects in their sensitivity to heavy metals are well documented. In general, most Ephemeroptera are highly sensitive whereas Trichoptera and some Chironomidae are quite tolerant (Sprague et al. 1965; Winner et al. 1980; Leland et al. 1989; Clements 1991a). Accordingly, percent compositions of Ephemeroptera (Van Hassel and Gaulke 1986) and Chironomidae (Winner et al. 1980) have been recommended as potential indicators of metal impact. Recently, Plafkin et al. (1989) developed a rapid bioassessment protocol for streams and rivers based on several measures of community composition. The rationale for several of the recommended metrics is based on the observation that Ephemeroptera, Plecoptera, and Trichoptera are sensitive to contaminants whereas Chironomidae are tolerant. Although these generalizations hold for certain classes of pollution, particularly organic enrichment, results of the present investigation suggest that some caution is required when applying these metrics to metal-impacted streams. For example, in both experimental streams and field biomonitoring at the Clinch River, Tanytarsini chironomids were shown to be as sensitive to metals as Ephemeroptera (Clements et al. 1988, 1989b). Other researchers have reported that certain species of Trichoptera and Plecoptera are highly tolerant of metals (Warnick and Bell 1969; Specht et al. 1984) and among the first groups to recover at metal-impacted streams (Petersen and Petersen 1983; Chadwick et al. 1986; Roline 1988; Clements 1991a).

Because of these limitations, there is a need to develop community-level metrics specific for heavy metals. The index of community sensitivity described in this study was useful for identifying metal-impacted stations and has several advantages. The experimental procedure employed to estimate sensitivity to metals has been suggested as an environmentally realistic approach for determining community-level responses to contaminants (Clements et al. 1988, 1989a; Pontasch and Cairns 1991). Using this approach, sensitivity values for many taxa may be determined simultaneously. Because sensitivity values were obtained experimentally, the index was useful for objectively distinguishing among reference and impacted sites in the field. Previous studies using biotic indices (Chandler 1970; Hilsenhoff 1987) have obtained sensitivity estimates based on presence or absence of specific taxa at impacted field sites. This approach has been criticized because assignment of species to sensitive or tolerant classes is quite subjective (Herricks and Cairns 1982).

The approach described in this study may be employed to improve rapid bioassessment protocols. After species sensitivity values are determined, ICS values may be obtained from either qualitative or quantitative estimates of relative abundance. Thus, species sensitivity values may be integrated with rapid bioassessment procedures (e.g. Plafkin et al. 1989) to obtain estimates of heavy metals impact.

The most important potential application of the ICS is its ability to discriminate among impacted and nonimpacted stations in other systems, based only on estimates of community composition. To illustrate this, ICS values were calculated for metal-impacted streams located in Ohio, Colorado, and Montana. Elam's Run (Ohio) was impacted by Cu, Cr, and Zn (Winner et al. 1980), Silver Bow Creek (Montana) was impacted by Cu, Fe, and Zn (Chadwick et al. 1986), and the Arkansas River (Colorado) was contaminated by Cu, Cd, Pb, and Zn (W. H. Clements, unpubl. data). All stations were sampled using quantitative sampling techniques. ICS values were calculated based on the proportion abundance of Ephemerop-

TABLE 3. Number of taxa, number of individuals, and ICS values from all reference stations combined. Stations with values significantly less than reference station values are indicated below the mean.

	Number of taxa	Number of individuals	ICS
1986			
Mean (±SD)	16.4 (±2.5)	112.0 (±28.9)	0.43 (±0.07)
Impacted stations	8, 11, 13	8, 11, 13	8, 11, 13
1987			
Mean (±SD)	17.2 (±2.4)	339.0 (±61.3)	0.66 (±0.03)
Impacted stations	13, 13A, 13B, 15	13, 13A, 13B, 15, 16	13, 13A, 13B, 15, 16
1988			
Mean (±SD)	18.1 (±2.1)	134.0 (±34.9)	0.40 (±0.08)
Impacted stations	13, 13A	13, 13A, 13B, 15	13, 13A
1989			
Mean (±SD)	22.1 (±1.2)	403.5 (±163.7)	0.36 (±0.07)
Impacted stations	13	15	13

TABLE 4. ICS values calculated for Elam's Run (Ohio), Silver Bow Creek (Montana), and the Arkansas River (Colorado). Because upstream reference stations were not available at either Elam's Run or Silver Bow Creek, nearby streams (Shayler's Run and Mill and Willow Creek) were used as reference sites.

Study site	Station	Location (km downstream)	ICS
Elam's Run	Shayler's	Reference	0.40
(Winner et al. 1980)	1	0.4	−0.11
	2	0.8	−0.07
	3	1.0	−0.04
	4	2.4	−0.01
	5	3.4	0.09
Silver Bow Creek	Mill and Willow	Reference	0.33
(Chadwick et al. 1986)	1	3.0	−0.06
	2	8.0	−0.12
	3	19.0	−0.06
	4	28.0	0.09
	5	36.0	0.15
Arkansas River	1	Reference	0.56
(W. H. Clements,	2	Reference	0.53
unpubl. data)	3	0.2	0.23
	5	12.0	0.09
	7	20.0	0.31
	8	48.0	0.43

tera, Trichoptera, and Orthocladiini using mean sensitivity (s_i) estimates for these three groups obtained from the Clinch River (=0.90, 0.23, and −0.14, respectively). ICS values at reference sites ranged from 0.33 to 0.56 (Table 4). Values at downstream stations were within the range of expected values for impacted sites, based on results obtained from the Clinch River, and gradually increased with distance downstream from the source of metals. Using the lowest ICS value obtained from a reference station at the Clinch River (0.30) to separate impacted and nonimpacted stations, we conclude that all stations at Elam's Run and Silver Bow Creek were impacted and stations 3–5 at the Arkansas River were impacted. These assessments agree with those of Winner et al. (1980) and Chadwick et al. (1986) for Elam's Run and Silver Bow Creek, respectively. An ICS value of 0.30 to separate impacted and nonimpacted sites at the Arkansas River may not be appropriate, since other community-level parameters (number of taxa, number of individuals, and canonical discriminant analysis) indicated that station 7 was still moderately polluted (W. H. Clements, unpubl. data).

Finally, although this investigation focused on heavy metals, the general approach described in this study is applicable to other classes of contaminants. Pontasch and Cairns (1991) exposed benthic communities in experimental streams to an effluent containing a mixture of chlorides, ammonia, organics, and metals. The authors noted considerable variation in responses among taxa (e.g. mayflies were reduced, net-spinning caddisflies were unaffected, and Orthocladiini chironomids were stimulated in treated streams), suggesting that species-specific sensitivity values may be used to develop community indices for more complex effluents. Similar experiments exposing indigenous benthic communities to pesticides are currently being conducted (K. W. Pontasch, Department of Biology, University of Northern Iowa, Cedar Falls, IA 50614, pers. comm.). We suggest that standardization of these procedures for other classes of contaminants in other ecoregions will result in environmentally realistic estimates of effects, which may be employed to validate laboratory toxicity tests.

Limitations of the Index of Community Sensitivity

Although the results shown in Table 4 support the hypothesis that ICS values may be used to assess the degree of metal contamination in different streams, there are several potential problems that must be considered. First, sensitivity values were obtained from a single set of experiments by exposing a specific community of benthic invertebrates to Cu for only 10 d. Because of differences in sensitivity within taxonomic groups (e.g. Baetidae versus Tricorythidae within the Ephemeroptera), as well as differences between age classes, results of this study are limited. Second, metal-sensitivity estimates were obtained by exposing benthic communities only to Cu. Variation in acute and chronic toxicity among different metals is well documented and may limit the usefulness of our index. However, we feel that this variation does not pose a serious problem to the development of the ICS. We hypothesize that if dominant taxa within a region are ranked from most sensitive to least sensitive for one metal, the ranking for another metal will be similar. This hypothesis is supported by the fact that field responses of benthic communities to heavy metals are generally similar, regardless of which metals are present (Clements 1991a). Third, estimates of relative field abundance (p_i) at the Clinch River included in the ICS were obtained from artificial substrates. Selectivity of artificial substrates for certain taxa is well known,

and the substrates employed in this study were no exception. However, despite the selectivity of substrate-filled trays, most dominant taxa collected in natural substrate were also present in the trays (Clements 1991b). Using benthic data collected with a 0.1-m^2 Hess sample in 1986, an ICS value of 0.33 was calculated for station 2 at the Clinch River. Although this value is lower than the value from substrate-filled trays (0.49) during this year, it is within the range of values for Clinch River reference stations.

In summary, an index for comparing effects of metals among streams or for assessing impact in the absence of adequate reference sites is of obvious significance. Unlike measurements such as the number of taxa or number of individuals, which may vary tremendously among streams because of natural differences in habitat characteristics, variation in the ICS is more specific to metal pollution. We suggest that ICS values could be employed to characterize the degree of metal pollution at several streams within a region. However, for reasons noted above, we recommend caution when using sensitivity estimates for Clinch River benthic invertebrates to obtain ICS values for streams in other ecoregions. Experiments to measure metal sensitivity of organisms collected from streams in the Rocky Mountains are currently being conducted (W. H. Clements, unpubl. data). By obtaining s_i values for dominant taxa within an ecoregion, more appropriate estimates of the ICS for streams within that region may be obtained.

Acknowledgments

The field assistance of D. Hammerdorfer, G. Church, and K. Sappington is greatly appreciated. We are particularly indebted to V. Taylor and the staff of the Clinch River Plant for logistical support during all phases of this research. Comments by S. Luoama, K. Pontasch, P. Kiffney, C. Synder, and an anonymous reviewer on earlier drafts of this manuscript are greatly appreciated. Support for this research was provided in part by a Cunningham Dissertation Research Fellowship from Virginia Polytechnic Institute and State University, the Mining and Minerals Resources Research Institute, and the American Electric Power Service Corporation, Columbus, OH.

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Factors Affecting Sampling Variability of Freshwater Periphyton and the Power of Periphyton Studies[1]

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Morin, A., and A. Cattaneo. 1992. Factors affecting sampling variability of freshwater periphyton and the power of periphyton studies. Can. J. Fish. Aquat. Sci. 49: 1695–1703.

Estimates of periphyton abundance, biomass (as biovolume, chlorophyll, dry mass, or ash-free dry mass), and production were reanalyzed to examine the effect of the mean value, sampler size, type of substratum, and habitat on the variance of these estimates. There were strong relationships between the variance and the mean for all variables considered. Variability per sampling unit increased with sampler size for all variables except production and biovolume, which were both unaffected. Chlorophyll estimates were significantly less variable on artificial than on natural substrata, but this trend was not confirmed by other variables. For biovolume, the trend was reversed. Habitat affects periphyton variability only for some variables: production was more variable in streams than in lakes whereas chlorophyll was less variable in artificial streams than in any other habitat. Although periphyton tends to be less patchy than benthos, detection of small differences still requires a very intense sampling effort. Since the median replication is 3–10 in most published studies, only large differences can be detected with 95% probability, from twofold for the most precise variable (biovolume) to eightfold for the less precise variable (dry mass). Our ability to detect patterns in periphyton ecology is thus very limited.

Des estimations de l'abondance du périphyton, de sa biomasse (sous forme de biovolume, chlorophylle, masse sèche ou masse sèche sans cendre) et de sa production ont été réanalysées dans le but d'examiner les effets de la valeur moyenne, du calibre de l'échantillonneur, du type de substrat et de l'habitat sur la variance de ces estimations. Pour toutes les variables considérées, on a trouvé une forte corrélation entre la variance et la moyenne. La variabilité par unité d'échantillonnage augmentait avec le calibre de l'échantillonneur pour toutes les variables, à l'exception de la production et du biovolume, lesquels n'étaient pas affectés. Les estimations de chlorophylle étaient significativement moins variables sur les substrats artificiels que sur les substrats naturels, mais cette tendance n'a pas été confirmée par les autres variables. Dans le cas du biovolume, la tendance était inversée. L'habitat n'influe sur la variabilité du périphyton que pour quelques variables : la production était plus variable dans les cours d'eau que dans les lacs, tandis que la chlorophylle était moins variable dans les cours d'eau artificiels que dans les autres habitats. Même si le périphyton tend à être moins épars que le benthos, la détection de petites différences exige cependant un effort d'échantillonnage extrêmement soutenu. Parce que le nombre de répétitions est de 3 à 10 dans la plupart des études publiées, seules les différences importantes peuvent être détectées avec une probabilité de 95 %, d'un facteur de deux pour la variable la plus précise (biovolume) à un facteur de huit pour la variable la moins précise (masse sèche). Notre capacité de détection des profils de l'écologie du périphyton est par conséquent très limitée.

Received August 27, 1991
Accepted February 11, 1992
(JB202)

Reçu le 27 août 1991
Accepté le 11 février 1992

The importance of periphyton, the community of attached algae in streams and lake littoral zones, is increasingly recognized. In natural communities, periphyton contributes significantly to the primary production (Minshall 1978; Cattaneo and Kalff 1980) and represents a readily available food for many invertebrates (Lamberti and Moore 1984) and fish (Power et al. 1985). In stressed systems, this community is ideal for studies of pollution impact because it is sessile and has a rapid rate of growth (Stevenson and Lowe 1986).

Periphyton studies often require a precise quantification that is made difficult by the notorious variability of this community (Weitzel et al. 1979). Highly aggregated distributions characterize other benthic communities, and general relations predicting variability have been calculated for lake (Downing 1979), stream (Morin 1985), and marine (Vézina 1988) benthos and for aquatic macrophytes (Downing and Anderson 1985). No comparable equations exist for periphyton. These relationships are useful in designing sampling programs, since the number of replicates necessary for a given precision can be calculated when the variability of the estimates is known. Variability can also be estimated by preliminary surveys (Cochran 1977), but these are costly and generally too small to yield reliable estimates of the number of replicates. As a result, many periphyton studies lack the power they need to answer the questions they claim to address.

In this study, we analyze many published and unpublished data to establish the relationship between the mean and variance of the variables that are most frequently used to describe periphyton: cell density, biovolume, chlorophyll, dry mass, ash

[1]Contribution 386 of the Groupe de recherches en Écologie des Eaux douces, Université de Montréal.

TABLE 1. Summary of the data obtained from the literature and our unpublished work that was used in this study.

Parameter	Ash-free dry mass ($g \cdot m^{-2}$)	Biovolume ($mm^3 \cdot m^{-2}$)	Chlorophyll ($mg \cdot m^{-2}$)	Dry mass ($g \cdot m^{-2}$)	Density ($cells \cdot m^{-2}$)	Production ($mg\ C \cdot m^{-2} \cdot d^{-1}$)
Median	5.4	1719	10.9	6.6	1.9×10^9	386
Minimum	0.02	1.5	0.01	0.03	1.0×10^8	0.6
Maximum	79	88 000	2385	46	2.3×10^8	2510
n	187	94	1194	57	55	200
Median area (cm^2)	17	138	16.5	47	200	0.64
Range of areas (cm^2)	4–300	1.7–200	1.1–819	7.8–200	2.2–200	0.13–25.5
Median no. of replicates	3	3	5	10	5	3
Range of replication	3–35	3–5	2–38	3–20	3–6	2–15

free dry mass, and production. We also examine if variability of periphyton is affected by the sampler size, the use of natural or artificial substrata, and whether the measurements were made in river, lake, or experimental habitats. We then evaluate the implications of these relationships for sampling design in periphyton studies. We calculate the number of replicates required for statistical comparison of abundance by a t-test at various power levels. Finally, in view of these results, we evaluate the power of most published periphyton studies and suggest the best general data transformation for parametric statistical analysis of these estimates.

Methods

Data Collection

Twenty journals, published between 1975 and 1990, were systematically scanned to collect the data (*Annales de limnologie*, *Aquatic Botany*, *Archiv für Hydrobiologie*, *Australian Journal of Marine and Freshwater Research*, *British Phycological Journal*, *Canadian Journal of Botany*, *Canadian Journal of Fisheries and Aquatic Sciences*, *Ecology*, *Freshwater Biology*, *Hydrobiologia*, *International Revue der gesamten Hydrobiologie*, *Journal of the North American Benthological Society*, *Journal of Phycology*, *Limnology and Oceanography*, *Naturaliste Canadien*, *New Zealand Journal of Marine and Freshwater Research*, *Oecologia*, *Oikos*, *Transactions of the North American Microscopical Society*, and *Verhandlungen Internationale Vereinigung für Theorische und Angewandte Limnologie*). We also included unpublished measurements made by the authors in lakes and streams of eastern Canada (nine separate studies) and northern Italy (two studies). Estimates of mean abundance, biovolume, chlorophyll, ash-free dry mass, dry mass, and production of periphyton and their spatial variance were extracted from tables and figures or calculated from raw data. We rejected estimates that included temporal variability in periphyton. For mean and variance pair, we recorded the habitat of origin (lake, river, experimental lake, or river), whether the measurements came from natural or artificial substrata and what type of substratum was sampled, how many replicates were taken, and what surface area was sampled by each replicate. When the surface area varied among replicates, for example when natural rocks were collected, we recorded the average size of the rocks sampled. Our unpublished data yielded 593 estimates of mean and variance of periphyton chlorophyll (out of 1194), 28 of periphyton ash-free dry mass (out of 187), and 18 of periphyton dry mass (out of 57).

Data Analysis

To estimate the relationship between the variance (s^2) and the mean (M) and to quantify the effect of sampler size (A, in square centimetres) on spatial variance, data were fitted by multple linear regression to the model

$$(1)\quad \log_{10} s^2 = a + b\log_{10} M + c\log_{10} A$$

where a, b, and c are fitted constants. Residual values (observed minus predicted values) for different sampling methods and habitats were then averaged and the 95% confidence intervals for these averages visually compared to detect differences in variability among methods or habitats after correction for the effects of the mean and sampler size.

Power Analysis

The multiple regression equations predicting spatial variance from mean and sampler size were used to estimate the number of replicates necessary to detect differences between means by a t-test for normal populations with unequal variances ("Welch's approximate t," Zar 1984, p. 131). The number of replicates required (n) to have a $(1 - \beta)$ probability of detecting a difference between two means that is significant at the α probability level was calculated by iteratively solving the equation

$$(2)\quad n \geq \frac{s_1^2 + s_2^2}{\delta^2}\left(t_{\alpha(2),\upsilon} + t_{\beta(1),\upsilon}\right)^2$$

where υ is calculated as

$$\upsilon = \frac{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)^2}{\frac{\left(\frac{s_1^2}{n_1}\right)^2}{n_1 - 1} + \frac{\left(\frac{s_2^2}{n_2}\right)^2}{n_2 - 1}}$$

and where $s^2{}_1$ and $s^2{}_2$ are the variances for the two means to be compared, δ is the absolute value of the difference between the two means to be compared, $t_{\alpha(2),\upsilon}$ is the critical value of the t-distribution with υ degrees of freedom for a two-tail test at the α level of probability, $t_{\beta(1),\upsilon}$ is the critical value of the t distribution for a one-tail test at the β level of probability (Zar 1984, p. 134), and n_1 and n_2 are the sizes of the samples on which the two means to be compared are calculated.

Results and Discussion

Description of the Data Sets

Chlorophyll was the periphyton descriptor most commonly reported, while density, dry mass, and biovolume were the least

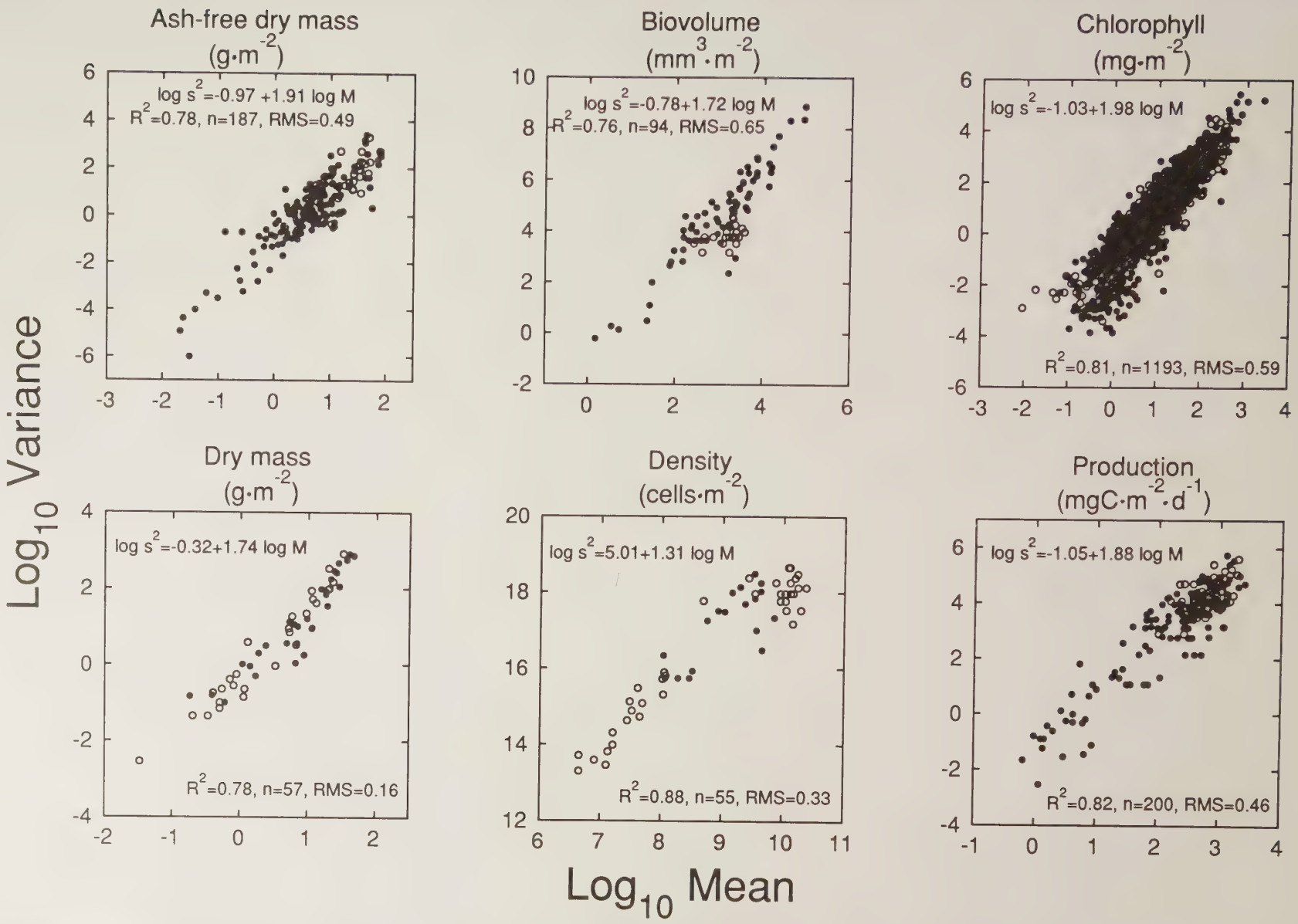

FIG. 1. Mean:variance relationships for various freshwater periphyton variables. Data include natural (○) and artificial (●) substrata. The equations for the simple linear regression fitted to all data are listed on each plot with the coefficient of determination (R^2), the number of observations (n), and the residual variance (RMS). Data sources: Bothwell and Jasper 1983; Bothwell et al. 1989; Burkholder and Wetzel 1990; Capblancq 1973; Carrick and Lowe 1988, 1989; Clark et al. 1979; Coleman and Dahm 1990; Crossey and La Point 1988; Dilks and Meier 1981; Fairchild and Lowe 1984; Fairchild et al 1985; Feminella et al. 1989; Flint and Goldman 1975; Goldsborough and Robinson 1986; Goldsborough et al. 1986; Graham 1990; Hansson 1990; Hoagland 1983; Howell 1990; Jónsson 1987; Kaufman 1980; Lamberti et al. 1989; Lamberti and Resh 1983a, 1983b, 1985; LaPerriere et al. 1989; Marks and Lowe 1989; Mason and Bryant 1975; Meier et al. 1983; Moore 1977; Murphy 1984; Palumbo et al. 1987; Peckarsky and Penton 1990; Perrin et al. 1987; Pringle 1987; Pringle et al. 1986; Rosemarin 1975; Schoenberg and Oliver 1988; Scrimgeour and Winterbourn 1989; Singh and Gaur 1989; Steinman and Lamberti 1988; Steinman et al. 1989; Steinman and Parker 1990; Stewart 1987; Swamikannu and Hoagland 1989; Van Raalte and Valeila 1976; Weitzel et al. 1979.

TABLE 2. Multiple regression models predicting sampling variance from mean abundance (M, units as in Table 1) and area (A,in cm^2) (Eq. 1). Listed are the fitted coefficients and their standard errors,the partial R^2, the probability associated with each coefficient (p), the number of observations (n), the multiple coefficient of determination (R^2), and the residual variance. ns denotes $p > 0.05$.

Dependent variable	Independent variable	Coefficient	Standard error	Partial R^2	p	n	R^2	Residual variance
Ash-free dry mass	Intercept	−1.56	0.16			187	0.80	0.45
	$\log_{10} M$	1.91	0.07	0.78	0.0001			
	$\log_{10} A$	0.47	0.11	0.02	0.0001			
Biovolume	Intercept	−0.78	0.31			94	0.76	0.65
	$\log_{10} M$	1.72	0.10	0.76	0.0001			
	$\log_{10} A$				ns			
Chlorophyll	Intercept	−1.68	0.06			1193	0.84	0.50
	$\log_{10} M$	2.07	0.03	0.81	0.0001			
	$\log_{10} A$	0.45	0.03	0.03	0.0001			
Dry mass	Intercept	−0.75	0.20			57	0.92	0.15
	$\log_{10} M$	1.81	0.08	0.91	0.0001			
	$\log_{10} A$	0.26	0.12	0.01	0.03			
Density	Intercept	5.48	0.60			55	0.89	0.30
	$\log_{10} M$	1.31	0.06	0.88	0.0001			
	$\log_{10} A$	−0.26	0.10	0.02	0.01			
Production	Intercept	−1.06	0.15			200	0.83	0.46
	$\log_{10} M$	1.88	0.06	0.83	0.0001			
	$\log_{10} A$				ns			

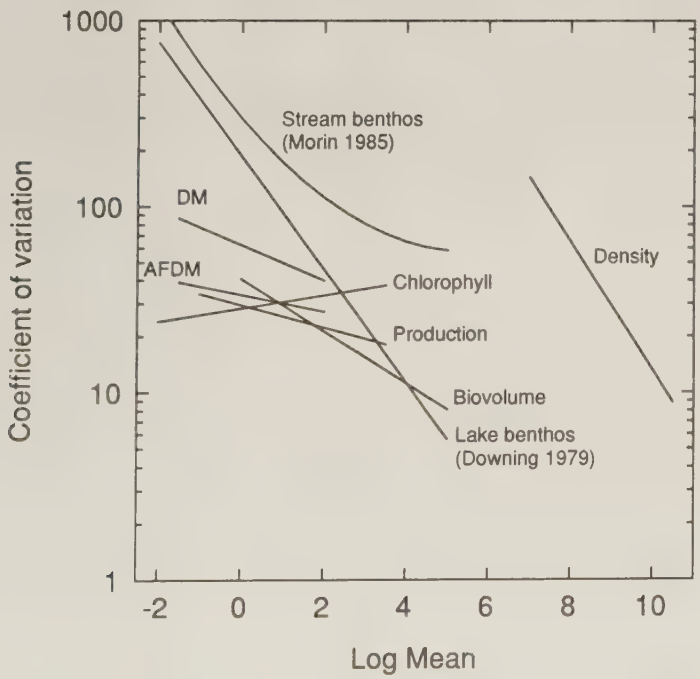

FIG. 2. Coefficient of variation as a function of the mean for freshwater periphyton and benthos. The coefficients of variation were calculated from the equation in Table 2 for a sampler of 20 cm^2 over the range of means for which data were available. Note that estimates of periphyton variables are less variable than estimates of benthos abundance. Densities of benthic organisms are in individuals·m^{-2} and units for the periphyton variables are as in Table 1.

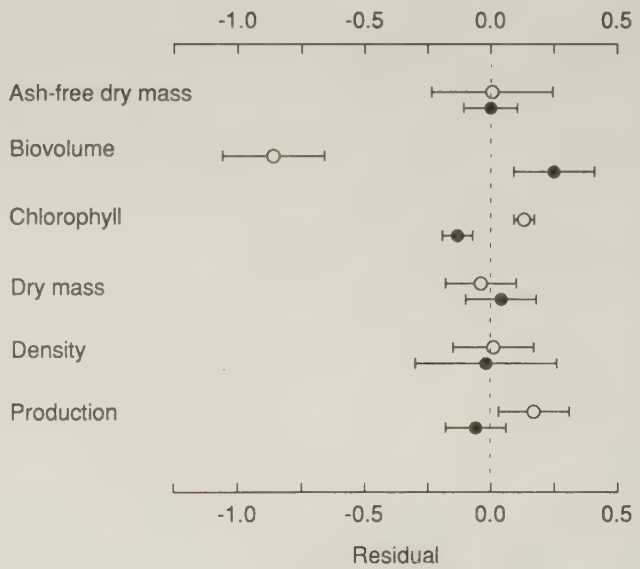

FIG. 3. Mean residual values for natural (○) and artificial (●) substrata. Negative and positive values indicate that the equations from Table 2 overestimate or underestimate variability, respectively. The bars indicate the 95% confidence interval for the mean.

popular (Table 1). Ash-free dry mass and production were intermediate. In general, researchers use few replicate measurements when they want to estimate periphyton abundance, biomass, or production (median = three to five replicates for all indices but dry mass). The median sampler size varies over 100-fold among the periphyton variables measured. In this data set, larger areas were sampled when optical measurements (density and biovolume) were made, while production estimates were on average performed on the smallest areas.

Relationship between the Variance and the Mean

Variance of periphyton variables increases with abundance. There were strong positive correlations between spatial variance and mean for all six periphyton variables (R^2 from 0.76 to 0.88, $p < 0.0001$; Fig. 1). The slopes of the relationships for ash-free dry mass, chlorophyll, and production were not significantly different from 2, implying that the standard deviation increases linearly with the mean and therefore that the coefficient of variation is independent of the mean and approx-

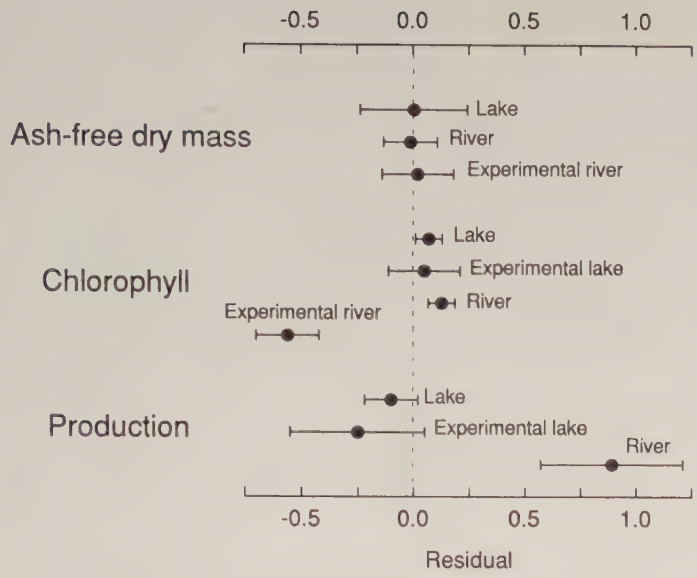

FIG. 4. Mean residuals for the models of Table 2 for each habitat. The bars indicate the 95% confidence interval for the mean. Data for natural and artificial substrata have been pooled to calculate the means.

imately constant. However, for density, biovolume, and dry mass the slopes of the relationships between the logarithm of the variance and the logarithm of the mean were significantly smaller than 2.0. This implies that the coefficient of variation for these measurements decreases with increasing mean (Fig. 2).

The strong relationships between variance and mean are similar to those observed for benthic organisms in lakes (Downing 1979), streams (Morin 1985), and oceans (Vézina 1988), although the periphyton slopes tended to be higher than those for benthic animals. Comparison of the variability of periphyton and benthos variables (Fig. 2) suggests that, in general, periphyton is less patchy or less aggregated than freshwater benthos, especially at low standing stock. Except for density estimates and biovolume, precision of periphyton estimates (denoted, for example, by the coefficient of variation) varies little as the mean changes. For periphyton density, the precision of the estimates increases with increasing abundance, as does the precision associated with abundance of zoobenthos.

Effect of Sampler Size

In general the variance of periphyton variables appeared to increase with sampler size, although spatial variance of density estimates decreased with increasing sampler size and sampler size had no effect on the variance of biovolume and production estimates (Table 2). Although sometimes significant, the effect of sampler size on variance accounted for only 0–3% of the variability in log variance, which is much less than the effect of the mean (76–91% of variability). However, the possibility remains that the positive correlation between variability and sampler size is the reflection of a tendency to sample more heterogeneous habitats when using larger samplers. Unfortunately there are no data yet available to examine this possibility.

Studies on sampling variability of freshwater benthic invertebrates and macrophytes also described a negative relationship between variance and sampler size (Downing 1979; Downing and Anderson 1985; Downing and Cyr 1985; Morin 1985). Vézina (1988) argued that this negative relationship is logically necessary when data are normalized per unit area rather than by sampling units and that sampler size should not affect variance per sampling unit. Reanalysis of periphyton data with means and variances expressed per sampling unit rather than per unit area shows that variance per sampling unit of ash-free dry mass, chlorophyll, dry mass, and density still increases with sampler size. The coefficient of sampler size (c in Eq. 1) varies from 0.37 to 0.56 when mean and variance are expressed per sampling unit, implying that variance per sampling unit increases about two- to threefold with a 10-fold increase in sampler size. Variance of biovolume and production is unaffected by sampler size when data are standardized per sampling unit. In general, the data suggest a significant positive effect of sampler size on sampling variance, since this trend is observed for four of the six periphyton variables studied here.

This does not necessarily invalidate Vézina's argument because at least two factors may create an artifactual positive correlation between variability and sampler size: subsampling that introduces measurement errors and a tendency to sample more heterogeneous habitats when larger samplers are used. Periphyton measurements made from larger samplers more often involve subsampling. Since Biggs (1987) found that subsampling can introduce measurement errors that are almost of the same order of magnitude as the variability between replicates that is commonly observed (coefficient of variation of 5–40%), the increase in periphyton variability with increasing sampler size could result from the introduction of subsampling variability. Authors seldom describe the extent of the area that was actually sampled during their study. Although most of the data analysis performed assumes random sampling, the possibility exists that researchers using small samplers sample more homogeneous and smaller areas than those using large samplers. Such a tendency, if it exists, could create the pattern observed between variability and sampler size.

Periphyton Variability on Natural and Artificial Substrata

Artificial substrata are often used to reduce periphyton variability and consequently the number of replicates necessary for a given level of precision. Some investigators hae confirmed this advantage (Tuchman and Stevenson 1980; Meier et al. 1983), but others have rejected it (Lowe and Gale 1980); Weitzel et al. 1979). Analyses of the residuals of the multiple regression models predicting sampling variance for the various parameters (Table 2) allow more general tests of this hypothesis.

Chlorophyll variability is indeed significantly lower for periphyton growing on artificial substrata than on natural substrata (Fig. 3). However, this pattern is not consistent among the other variables examined. In the case of biovolume, variability is significantly lower on the natural substrata. The results for chlorophyll, based on a larger sample, should be the most robust because they are less likely to be biased by the peculiarity of some studies. However, such tests are not powerful because the estimates are from different sites, habitats, or seasons so that significant differences in variability may be too small to be detected in a general comparison. Indeed, such a comparison of stream benthos variability on artificial and natural substrata failed to show a significant difference (Morin 1985) that was detected in a more controlled study (Morin 1986). Therefore, it remains possible that the use of artificial substrata reduces sampling variability; however, the apparent gain in precision is small and may be offset by the bias due to the selectivity of artificial substrata, as was observed by Morin (1986) for stream benthos.

Periphyton Variability in Different Habitats

The easiest explanation for high variability in the abundance and activity among periphyton samples is high habitat diversity.

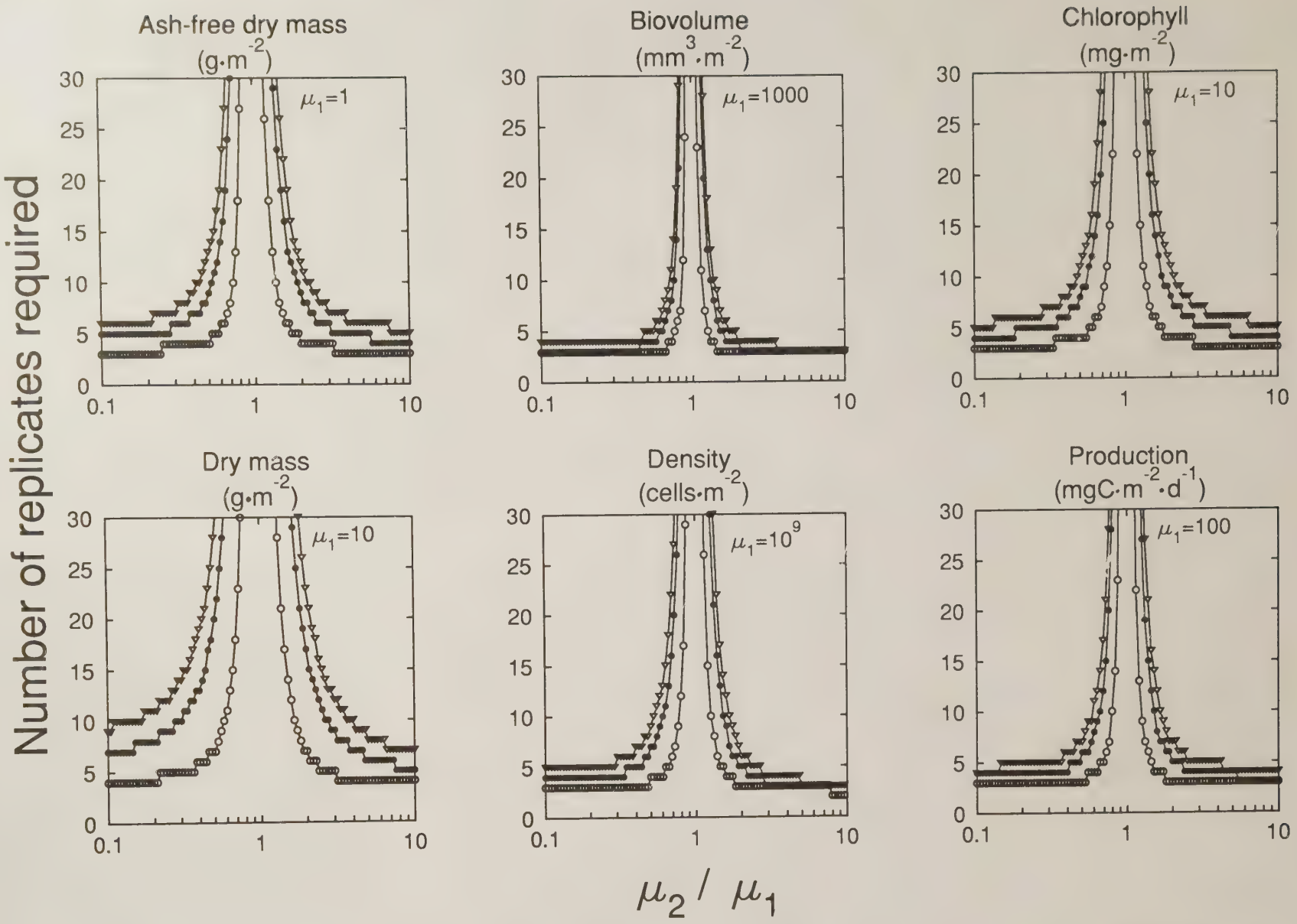

FIG. 5. Sampling effort needed to have a given probability of detecting differences between two means by a two-tail *t*-test for normal populations with unequal variances. Number of replicates required to detect a difference (significant at the $\alpha = 0.05$ level) between two means (μ_1 and μ_2) with power ($1 - \beta$) equal to 0.5 (◯), 0.95 (●), and 0.99 (▽). These figures were obtained by solving Eq. 2, using the equations given in Table 2 for sampling variance at the median sampler size listed in Table 1. Note the very slight asymmetry of these figures caused by the nonlinear relationship between the standard deviation and the mean. These figures can be used to indicate how many replicates should be collected to perform a comparison between two sites or treatments. For example, if the ash-free dry masses of periphyton to be compared in two habitats are 1 and 2 g·m^{-2}, then the ratio μ_2/μ_1 is 2.0, and four replicates would be required to have a 50% probability of detecting a significant difference between these two means. A rough estimate of the required sampling effort for any two means can be read from the ratio μ_2/μ_1.

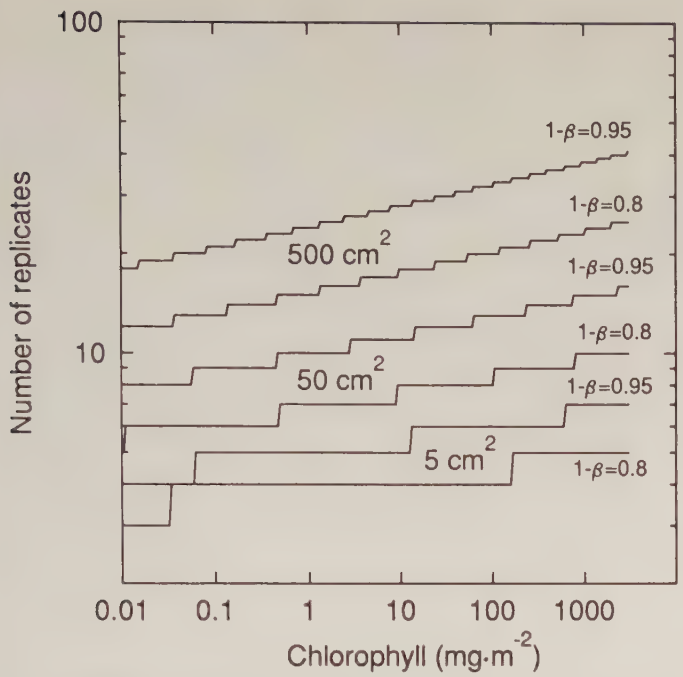

FIG. 6. Number of replicates required to detect a 50% reduction in chlorophyll (significant at the $\alpha = 0.05$ level) with samplers of different sizes (A, in cm^2) at two power levels ($1 - \beta$). Note that required sample sizes increase with increasing sampler area.

Differential flow around the uneven substratum of stream beds may create many microenvironments and more patchy benthic distributions than found in the relatively homogeneous lake littoral (Morin 1985). To escape the effects of habitat variability or to apply replicated treatments, periphyton is frequently and increasingly measured in experimental systems, limnocorals in lakes, and artificial channels built inside or outside natural streams, because the substrata and the physicochemical conditions are presumed to be more homogeneous in these experimental systems than in the natural habitats. To determine if these obvious differences in habitat heterogeneity affect the variability of periphyton in different habitats, we again analyzed the residuals of the multiple regressions in Table 2. The analysis is limited to production, ash-free dry mass, and chlorophyll, since insufficient data were available for the other variables.

The hypothesis that the variability is higher in natural streams is only partially confirmed. Production estimates are significantly more variable in streams than in lakes, but no such difference is observed for the two measures of standing crop variables (ash-free dry mass and chlorophyll; Fig. 4). Periphyton variability does not appear different in natural lakes versus experimental lake systems, but chlorophyll estimates in artificial streams are significantly less variable than those in natural streams. In contrast, ash-free dry mass estimates show similar variability across all the habitats sampled.

At least for chlorophyll, higher precision can be obtained using artificial streams. Although this smaller variability implies fewer replicate measurements, it should also warn that these systems fail to reproduce heterogeneity of the natural habitat. To the extent that this heterogeneity is important to stream function, artificial streams are likely to misrepresent the natural systems.

Data Tansformations

Parametric statistical analyses generally require equality of variances among treatments or sites. The strong dependence of variances of periphyton descriptors on their means implies that most data sets violate this assumption of homoscedasticity and that proper analysis demands transformation of data. The variance in Y can be stabilized (Green 1979) by transforming Y to Y' as

$$(4) \quad \begin{aligned} Y' &= Y^{1-b/2} && \text{if } b \neq 2 \\ Y' &= \log(Y) && \text{if } b = 2 \end{aligned}$$

where b is the slope of the regression relating the log variance to the log mean. Since the slope of the mean:variance relationship for periphyton variables, except density, is close to 2, the logarithmic transformation should stabilize the variance in most cases. For density estimates, however, the third-root transformation should be more appropriate. We nevertheless encourage researchers to examine their data for the appropriate transformation, since the pattern seen here from a wide range of values may not hold on the narrower ranges that are typical of most field studies.

Sampling Design and Power Analysis

The strong dependence of sampling variance on the mean and the sampler size can be put to use in designing experiments or surveys dealing with periphyton. The relationships depicted by Fig. 1 and Table 2 can be inserted into Eq. 2 to express the number of replicates requried as a function of the means to be compared (μ_1 and μ_2) and the sampler size (A, in square centimetres):

$$(5) \quad n \geq \frac{10^a A^c (\mu_1^b + \mu_2^b)}{(\mu_1 - \mu_2)^2} (t_{\alpha(2),\upsilon} + t_{\beta(1),\upsilon})^2$$

where a, b, and c are the coefficients of Eq. 1 fitted to the data (see Table 2). Figures 5 and 6 illustrate solutions of Eq. 5 for various periphyton variables.

Required sampling effort increases exponentially as the difference between the means to be compared decreases and increases with increasing desired probability of detecting a significant difference (Fig. 5). Differences in the variability of periphyton descriptors (Fig. 2) explain the differences in required effort among periphyton variables in Fig. 5. Biovolume, production, and density require less replication than ash-free dry mass, chlorophyll, and dry mass.

Assuming that the observed positive correlation between sampling variability and sampler size is reflecting a real tendency and is not an artifact of additional variability included by subsampling or sampling more heterogeneous habitats when large samplers are used, small sampler sizes appear the most efficient when periphyton is measured as chlorophyll (Fig. 6). Because the variance of chlorophyll estimates increases with sampler size in our data set, the number of replicates required to detect a 50% reduction in chlorophyll concentration roughly doubles when sampler size is increased 10-fold. The effect of sampler size is slightly more pronounced for ash-free dry mass, but less acute or absent for biovolume, dry mass, or production. For density estimates, large samplers will yield more precise results for a given number of samples. This suggests that smaller samplers are to be preferred because they yield more or equally precise estimates of standing stock or production and in addition they disturb the habitat less.

Current study designs imply that field studies will only detect differences in periphyton abundance or productivity where the means differ by a factor of 2 or more. Small differences in periphyton variables would require a very intense sampling effort: typically, n should exceed 20 to have a 95% probability

of detecting a 25% difference that would be significant at the 5% level. Such effort is rarely reported in published studies (median replication is 3–10). Depending on the periphyton variable sampled, means have to differ by a factor ranging from 2 (biovolume) to 8 (dry mass) to have a 95% probability of being declared significantly different when five replicates are taken. The above power and optimal design calculations do not consider the effects of data transformation, but general patterns and conclusions are unlikely to be affected by transformation. Similar required sample sizes for stated power and detectable differences for a fixed sample size were obtained using our unpublished transformed raw data.

Studies examining the effects of factors influencing periphyton are likely to detect important factors statistically only if the magnitude of the difference is large, unless sampling effort is much higher than usual. The low discriminating power of the majority of published studies dealing with periphyton could explain several apparent contradictions and some negative results. The traditionally low replication in periphyton studies also prevents the detection of the effectiveness of management practices or the ravages of pollution until the effects are large.

Acknowledgements

This research was funded by NSERC operating grants to Antonella Cattaneo and Antoine Morin and the Challenge Program for student summer employment of Canada Ministry for Youth. We thank M. C. Amireault for her diligent bibliographic research. R. H. Peters commented on an earlier draft.

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Changes in the Olfactory Function of Atlantic Salmon, *Salmo salar*, in the Course of Smoltification

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Morin, P.-P., and K. B. Døving. 1992. Changes in the olfactory function of Atlantic salmon, *Salmo salar*, in the course of smoltification. Can. J. Fish. Aquat. Sci. 49: 1704–1713.

Electrophysiological responses were recorded from the surface of the rostromedial and rostrolateral regions of the right olfactory bulb of Atlantic salmon, *Salmo salar*, during stimulation of the olfactory epithelium with different concentrations of L-alanine and taurocholate. Fish exposed to taurocholate prior to testing were compared with nonexposed fish. The results revealed significant week-to-week differences in olfactory activity in nonexposed fish, with two peaks in activity of similar magnitude. The first peak occurred at the beginning of smoltification, when olfactory sensitivity reached its maximum (low response threshold). The second peak occurred after smoltification, when sensitivity was roughly 10 times lower (higher response threshold) than during the first peak. Fish exposed to taurocholate prior to testing did not exhibit these peaks in olfactory activity. Marked changes in the spatial distribution of the electrophysiological response to L-alanine and taurocholate occurred throughout the smoltification process in nonexposed fish. The two peaks in olfactory activity that we identified coincide with two peaks in odor learning (Morin et al. 1989. Can. J. Fish. Aquat. Sci. 46: 122–130, 131–136); the first coincides with a unique capacity for odor memory and is apparently a neural manifestation of a sensitive period for olfactory imprinting in Atlantic salmon.

Les réponses électrophysiologiques sont enregistrées à la surface des régions rostromédiale et rostrolatérale du bulbe olfactif droit du saumon atlantique, *Salmo salar*, lorsque l'épithélium olfactif est stimulé à différentes concentrations de L-alanine et de taurocholate. Les poissons exposés à la taurocholate avant le test électrophysiologique sont comparés à des poissons nonexposés. Chez ces derniers, des différences significatives d'activité olfactive sont apparues, de semaine en semaine, en mettant en évidence deux pics d'activité d'ampleur similaire. Le premier pic est apparu au début de la smoltification lorsque la sensibilité olfactive était maximale (seuil bas). Le deuxième pic est apparu après la smoltification lorsque la sensibilité était environ 10 fois plus basse (seuil élevé) que lors du premier pic. Les poissons exposés à la taurocholate avant le test électrophysiologique n'ont pas atteint ces pics d'activité olfactive. Des changements marqués dans la distribution spatiale de la réponse électrophysiologique à la L-alanine et à la taurocholate sont apparus tout au long de la smoltification. Les deux pics d'activité olfactive que nous avons identifiés coincident à deux pics d'apprentissage olfactif (Morin et al. 1989. J. can. sci. halieut. aquat. 46: 122–130, 131–136); le premier pic coincide à une capacité unique de mémoire olfactive et est apparemment la manifestation neurale d'une période sensible d'empreinte olfactive chez le saumon atlantique.

Received April 26, 1991
Accepted February 7, 1992
(JB016)

Reçu le 26 avril 1991
Accepté le 7 février 1992

Nordeng (1971, 1977) proposed that in anadromous fishes, juvenile conspecifics that reside in freshwater and smolts that migrate to sea both release population-specific odors (pheromones) that are used by mature adults as cues to guide them to their home stream. Related to this "pheromone hypothesis" is the idea that population-specific pheromones are innately recognized by migrating adults (Nordeng 1989). An alternative to the pheromone hypothesis, put forward and elaborated by Hasler and co-workers (see Hasler and Scholz 1983 for review), is the "imprinting hypothesis", which states that recognition of the home stream results from a relatively rapid odor learning process during a sensitive period in the smoltification process. It was initially proposed that young salmon learned to recognize scents emanating from the soil and flora in the drainage basin of the natal stream (Hasler and Wisby 1951). However, within the context of more classical examples of natural imprinting among higher vertebrates whereby young animals learn the physical characteristics of a family member (Hess 1973), potential learning of cues produced by conspecifics may also be considered in salmon imprinting. Evidence supporting both the imprinting and the pheromone hypotheses has accumulated over the last two decades (Hasler and Scholz 1983; Smith 1985), with each having been questioned from time to time (Stabell 1984; Quinn and Courtenay 1989).

The adaptive significance of variations in life history among local populations of anadromous salmonids was emphasized by Schaffer and Elson (1975), who argued that such variations may maximize the reproductive fitness of fish in differing environmental conditions. Variations in the use of olfactory cues by homing salmon may also exist to optimize recognition of the home stream and migration upstream toward spawning grounds. Hassler and Kucas (1988) have produced some evidence that anadromous salmonids use various cues and/or various mechanisms to detect their home stream. They showed that when the site of release is scented with morpholine, coho salmon (*Oncorhynchus kisutch*) that were exposed to morpholine during smoltification and released from a hatchery into its

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supporting river returned in significantly larger numbers than fish that were not so exposed. Thus, the returning migrants from the control nonexposed group normally responded to the natural cues originating from the hatchery and release site, while those from the morpholine-exposed group also responding to these cues were able to learn an additional cue through imprinting to direct their migration toward their site of release.

A study on Atlantic salmon, *Salmo salar*, conducted by Morin et al. (1989a) showed two peaks of a conditioned cardiac response to an odorant (odor learning) during smoltification, the first of which the authors speculated was one manifestation of an olfactory imprinting process. In this study, the conditioned heart rate response to L-cysteine was significantly greater in juvenile salmon when testing occurred between 21 and 28 d (first 8-d peak) and between 61 and 68 d (second 8-d peak) after the onset of induced smoltification. Other nonconditioned fish that were continuously exposed to L-cysteine for an 8-d period during smoltification developed an odor memory to L-cysteine only if they had been exposed between 21 and 28 d after the onset of smoltification, the 8-d period coinciding with the first peak of odor learning in the conditioned fish. According to Morin et al. (1989a), the first peak in odor learning during smoltification is associated with a sensitive period for olfactory imprinting (SPOI). These authors also suggested that olfactory sensitivity may increase concomitantly with olfactory learning during smoltification. In addition, Morin et al. (1989b) reported significant correlations between olfactory learning ability and thyroid activity shown histologically in the thyroid gland during smoltification. Increased thyroid activity during smoltification has been shown to be associated with increased sensitivity to various external stimuli (Hoar 1953; Baggerman 1960; Saunders and Henderson 1970; Fontaine 1975).

There is virtually no specific information on changes in olfactory sensitivity during smoltification. Rehnberg et al. (1985) assessed odor sensitivity in coho salmon by measuring an avoidance response to amino acids (L-serine, and D- or L-alanine) in parr and smolts in a two-choice Y-maze. For each odorant, they found a higher threshold concentration (10^{-5} or 10^{-6} M) for odor detection in fish tested during or after smoltification as compared with fish tested before smoltification (10^{-7} M). However, these results should be interpreted with caution because Rehnberg et al. (1985) did not specify exactly when during smoltification the fish were tested, and they apparently pooled data obtained throughout the period of smoltification.

In order to assess neural changes in the olfactory system of salmon during smoltification, we designed an experiment with three objectives. The first objective was to investigate neural responses during the smoltification-associated SPOI (Morin et al. 1989a) by measuring the electrophysiological (EEG) activity in the olfactory bulb of young salmon following stimulation of the olfactory epithelium with either L-alanine or taurocholate over a 10-wk experimentation period. We chose L-alanine and taurocholate because they are highly stimulatory to the olfactory system of salmonids (Sutterlin and Sutterlin 1971; Hara 1973; Døving et al. 1980). According to Bateson (1964), the sensitive period begins when a stimulus becomes conspicuous to an animal. This increase in stimulus conspicuousness may be a function of heightened sensory sensitivity to a stimulus that would facilitate its detection and perhaps the learning of biologically significant events associated with it. Accordingly, we postulated that olfactory activity in terms of EEG response magnitude, as well as olfactory sensitivity in terms of response threshold, would be optimal during the SPOI.

The second objective was to compare the spatial distribution of the EEG response from the rostrolateral region of the olfactory bulb with that from the rostromedial region while fish were being stimulated with either L-alanine or taurocholate during the smoltification-associated SPOI. There is substantial evidence that amino acids evoke a stronger response in the lateral region of the bulb whereas bile salts evoke a stronger response in the medial region (reviewed by Døving 1989). Studies of the brains of young precocial birds have shown that changes occur in the neural substrate as a result of imprinting (Horn 1986). We therefore postulated that structural changes would occur in the neural network of the olfactory bulb in young salmon during the smoltification-associated SPOI and that these changes might have the effect of changing the pattern of distribution of the EEG response to L-alanine and/or taurocholate.

The third objective was to evaluate the effect of prolonged exposure of fish to a taurocholate-enriched environment on the EEG response to L-alanine or taurocholate during the smoltification-associated SPOI. Because structural changes are known to occur in the olfactory bulb of mammals following prolonged exposure to a single odor (Døving and Pinching 1973; Pinching and Døving 1974; Harvey and Cowley 1984; Rosselli-Austin and Williams 1990), it seemed reasonable to expect changes in the bulbar EEG response of fish after prolonged exposure to a single odorant. In the context of imprinting, stronger EEG activity may occur in the olfactory bulb of fish as a result of exposure to an odor during the SPOI. The only available olfactory data that demonstrate effects of such exposure on the ontogeny of EEG neural activity during imprinting have been obtained by Schmidt and Eckert (1988). They found that in mice, the EEG response to geraniol was significantly stronger in animals previously exposed to geraniol-enriched air from day 6 to day 12 after birth than in either nonexposed animals or animals exposed after birth from day 0 to day 6. The authors interpreted this specific neural response as evidence of a SPOI. Accordingly, we postulated that previous exposure to a taurocholate-enriched environment would increase the EEG response in the olfactory bulb of salmon during the smoltification-associated SPOI.

Materials and Methods

Fish and Maintenance

We used 2-yr-old juvenile Atlantic salmon with a mean fork length of 11.3 cm ± 0.7 SD at the start of the study. At the beginning of the 10-wk experimentation period, the age of fish since birth was 690 d. They were hatchery-raised offspring of a wild stock obtained from "Lierelva", a small river 30 km southwest of Oslo, Norway. Fish were transported to the aquarium facilities at the Department of Biology, University of Oslo, and held in groups of 50 in six identical 1-m-diameter tanks supplied with 6.4°C (range = 5.9–6.9°C) dechlorinated city water, under a constant 16 h dark : 8 h light photoperiod regime. Each tank was lighted by two 15-W bulbs situated 35 cm above the water surface. The light intensity was kept constant, but could vary from 1 to 30 lx depending on the position of the fish in the tanks. The mean water flow through each tank was 4.88 L/min. The fish were not fed during the initial 16-d acclimation period that preceded the experiment.

During the 10-wk experimentation period the fish were fed with ST40-3 dry food pellets (Ewos Aquaculture International) until satiated. They were fed twice daily during week 1 (09:00 and 14:00) and three times daily (09:00, 14:00, and 17:00) from week 2 to week 10.

Induction of Smoltification

Smoltification was induced at the beginning of week 2 by raising the temperature to 12.6°C (range = 11.6–13.6°C) and by increasing the photoperiod to 8 h dark : 16 h light. On the first day of week 2 the water temperature began to increase at 09:00 and reached 12.6 ± 1.0°C at 12:00. On the same day the 8-h light increment began at 16:00 so that the light photoperiod lasted until 24:00. Parr coloration was used as the index of smoltification (Morin et al. 1989a).

Experimental Design

During the 10-wk experimentation period, half of the fish (three tanks) were continuously exposed to a taurocholate-enriched environment. A known quantity of taurocholic acid sodium salt (Sigma Chemical Co.) was diluted in distilled water and metered slowly (1 mL/min) into each odor-exposed tank using two small dosing pumps. The final concentration of taurocholate in each tank was estimated to be 10^{-7} M.

Two fish per day were selected at random for EEG recordings. The first fish was tested at 09:30 and the second at 13:30. One of these fish had been exposed to the taurocholate-enriched environment prior to testing, while the other fish had not. The order of testing was determined at random. EEG recordings were made during 10 successive 5-d weeks. Thus, a total of 100 fish were tested. Fish were discarded after testing.

Experimental Procedure

The fish were tranquilized with chlorobutanol in seawater (80 mg/L), anesthetized intraperitoneally with amytal (30 mg/kg, Keys and Wells 1931), and paralyzed with tubocurarin chloride (2.5 mg/kg). Each anesthetized fish was wrapped in a wet cloth and held firmly in a cradle. The gills were perfused continually (0.3 L/min) with dechlorinated tap water (mean temperature = 9.1°C ± 1.5 SD). The roof of the skull was open and mesenchymal tissue removed to expose the dorsal surface of the two olfactory bulbs. The right olfactory epithelium was continuously perfused with standardized artificial pond water (0.4 mM Na^+, 0.04 mM K^+, 0.4 mM Ca^{2+}, 0.84 mM Cl^-, 0.4 mM HCO_3^-, pH = 7.6) (Thommesen 1982). Nasal water flow was kept constant at 6.6 mL/min ± 0.2 SD.

Anhydrous L-alanine (Sigma Chemical Co.) and taurocholic acid sodium salt diluted in the rinsing water (standardized artificial pond water) were used as olfactory stimuli. Each odorant was prepared in decadic aliquots, from 10^{-8} to 10^{-4} M. Presentation of the odorant was controlled manually by a stopcock. The odorant was delivered through a 10-mL glass pipette into the continuous flow of rising water over the olfactory epithelium. Because the olfactory system of salmonids is highly sensitive to odorants such as bile salts and amino acids (Døving 1989), great care was taken to avoid contamination. All glassware used for the preparation of odorants was thoroughly rinsed with methanol, followed by double-distilled water and rinsing water. Latex gloves were worn when preparing test solutions.

The order of presentation of taurocholate or L-alanine varied at random, with each odorant delivered in ascending concentrations. Three consecutive trials were carried out at each concentration of a given odorant. Three more consecutive trials using rinsing water instead of an odorant were carried out between the presentation of the first and second odorant. Before starting a trial, the glass delivery pipette was rinsed with the particular odorant at the same concentration to be tested. A trial began by switching on the stopcock until 2 mL of the odorant had drained from the pipette onto the right naris of the fish. The stopcock was then switched off. We waited 60 s between trials to avoid adaptation.

The olfactory epithelium was stimulated and EEG recordings made on the right olfactory bulb. Responses from the olfactory bulb were recorded simultaneously at two different positions: the rostromedial and rostrolateral regions of the bulb (Thommesen 1978). AC potentials were picked up by two Ag/AgCl electrodes coated with an insulating layer of varnish, leaving a 1-mm uncoated tip. The tip of each electrode was 105 μm in diameter.

Changes in potentials were fed to two differential preamplifiers with a frequency response of 0.1–30 Hz displayed on an oscilloscope and monitored on a DC tape recorder (Tandberg FM, series 100) and a pen recorder. All recordings were monopolar and the preparation was grounded through a common indifferent electrode that was placed on the chondrocranium just adjacent to the opening in the skull.

Blood flow in the brain surface vessels was checked regularly. Tissue was used as a wick to remove the excess fluid covering the bulb in the vicinity of the recording electrodes. This important procedure ensured proper contact between the surface of the bulb and the recording electrodes and minimized baseline recording differences from one preparation to another.

Evaluation of Responses and Analyses

AC potentials recorded on magnetic tape were digitized through an analog/digital converter connected to a PC microcomputer. Odor stimulation evoked a change in the activity of the olfactory bulb, an EEG response traditionally named an induced wave, the amplitude of which can be used as a measure of induced activity (Døving et al. 1980; Thommesen 1978). Under the conditions of the present study, these induced waves were small and sometimes difficult to measure. To avoid such ambiguity and to obtain objective measures of the responses, we chose to perform a numerical integration. The "energy" was operationally defined as the sum of the amplitudes squared, with a sampling rate of 1 kHz. To assess the effect of odor stimulation on the EEG activity, an "energy ratio" was calculated by dividing the energy value for the 2-s window during odor presentation by the energy value for the 2-s window preceding odor presentation. An energy ratio less than or equal to 1.0 indicates no odor response.

Four separate three-way analyses of variance (weeks × treatment groups × odorant concentrations) with repeated measures of odorant concentrations were carried out for EEG data collected from the lateral and medial regions of the olfactory bulb responding to either L-alanine or taurocholate. Thus, there was one analysis of variance for each of the following combinations of recording site and odorant: lateral–alanine, lateral–taurocholate, medial–alanine, and medial–taurocholate. The procedure MANOVA from SPSS-PC was used to analyze the gradual change in the relative distribution of the EEG response across the four combinations of recording site and odorant over the course of smoltification and to compare the distribution of the EEG response between pairs of consecutive 2-wk intervals (subcommand CONTRAST, SPSS-PC).

In each fish, and for each combination of recording site and odorant, a response threshold was estimated from the visual layout displayed on the computer monitor. The EEG response received a score of 1 when the amplitude was judged to be greater during than before an odor stimulation. A score of 0 was awarded if there was any doubt as to the existence of such a difference in amplitude of response. For each individual fish,

TABLE 1. Threshold variations to odorants in nonexposed (bold typeface) and exposed fish at the time of smoltification. Entries give the lowest threshold, in log molar concentrations, observed during a particular week and the digit in parentheses indicates the number of fish that displayed the threshold. The entry ">−4" means that no fish responded to a concentration of 10^{-4} M. Combinations of recording site and odorant are lateral–alanine (lateral–ala), medial–alanine (medial–ala), lateral–taurocholate (lateral–tar), and medial–taurocholate (medial–tar).

Recording site and odorant	Week									
	1	2	3	4	5	6	7	8	9	10
Lateral–ala	**-6(1)**	**-7(1)**	**-6(2)**	**-8(1)**	**-7(1)**	**-5(1)**	**-5(1)**	**-7(2)**	**-7(1)**	**-5(1)**
	-6(1)	-4(1)	-6(1)	-6(1)	-5(1)	-5(2)	-5(1)	-8(1)	-7(1)	-7(1)
Medial–ala	**-4(1)**	**-4(3)**	**-6(2)**	**-8(2)**	**-5(1)**	**-5(2)**	**-5(1)**	**-7(1)**	**-6(1)**	**-5(1)**
	-5(1)	-5(1)	-5(1)	-7(2)	-5(1)	-5(2)	-7(1)	-8(1)	-7(1)	-5(2)
Lateral–tar	**-5(1)**	**>-4**	**-4(1)**	**-7(3)**	**>-4**	**>-4**	**-5(1)**	**-8(1)**	**-6(2)**	**-6(1)**
	-6(1)	>-4	-7(1)	-7(1)	-6(1)	-8(1)	>-4	-7(1)	-6(2)	-8(1)
Medial–tar	**-5(1)**	**>-4**	**-4(1)**	**-8(1)**	**>-4**	**-5(1)**	**>-4**	**-6(1)**	**-7(1)**	**>-4**
	-7(1)	>-4	-5(1)	>-4	-4(1)	>-4	>-4	-6(1)	-6(2)	-7(1)

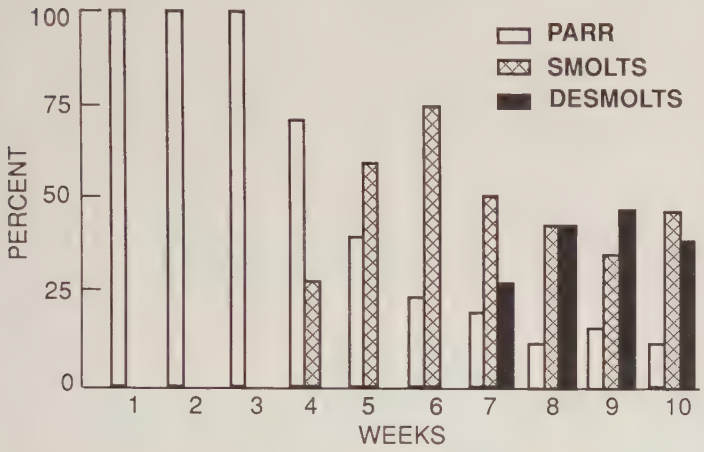

FIG. 1. Mean percent of parr, smolts, and desmolts in 2-yr-old Atlantic salmon. Fish were the hatchery-raised offspring of a wild stock from "Lierelva", Norway. Temperature was raised and photoperiod lengthened during week 2 of a 10-wk experimentation period to induce smoltification.

the criterion for determination of threshold was two out of three EEG responses with a score of 1. For each week of the 10-wk experimentation period, we chose the lowest threshold and counted the number of fish that reached the response criterion.

Results

Smoltification

The induction of smoltification began during week 2 of the 10-wk experimentation period when the water temperature increased from 6.6 to 12.6°C and the photoperiod from 8 to 16 h (Fig. 1). From week 2 until the end of the 10-wk period, water temperatrue and photoperiod remained constant. Judging from the external appearance of fish, the smoltification process lasted roughly 21 d. It began during week 4 when the first external signs of this process were observed in about 25% of the fish. Smoltification ended during week 6 when 75% of fish were classified as smolts. Signs of desmoltification (Evropeytseva 1963) were observed during week 7, with the reappearance of certain parr characteristics (e.g. contrasting dark black and yellowish belly) in about 25% of the smolts. The percentage of desmolts increased to about 40% toward the end of the 10-wk experimentation period. Less than 20% of the fish remained as parr throughout the experiment.

Weekly Changes in Olfactory Sensitivity

Visual assessment of the EEG response to L-alanine and taurocholate in both nonexposed and exposed fish allowed us to measure response thresholds and assess changes in olfactory sensitivity throughout the 10-wk experimentation period (Table 1). The lowest threshold before the fish showed signs of smoltification (weeks 1–3) was 10^{-7} M. During and after smoltification (weeks 4–10), the lowest response threshold was 10^{-8} M.

In nonexposed fish the strongest olfactory sensitivity occured during week 4, with a response threshold of 10^{-8} M when the lateral region of the olfactory bulb responded to L-alanine and the medial region of the olfactory bulb to L-alanine and taurocholate. There was also a higher response threshold at 10^{-7} M when the lateral region of the olfactory bulb responded to taurocholate. Thus, nonexposed fish that were tested during week 4 were more sensitive than nonexposed fish tested during any other week of the 10-wk experimentation period, including those tested during week 8. The lowest threshold of 10^{-8} M in nonexposed fish tested during week 8 was reached only when the lateral part of the bulb responded to taurocholate.

Differences in olfactory sensitivity throughout the 10-wk experimentation period were not so clearly evident in exposed fish. During week 10, the lowest response threshold of exposed fish was 10^{-8} M, when the lateral region of the olfactory bulb responded to L-alanine or taurocholate. However, when the medial region of the olfactory bulb responded to L-alanine, the response threshold of exposed fish was relatively high, 10^{-5} M. Exposed fish were equally sensitive when tested during week 6 or week 8. During week 6, the lowest response threshold in exposed fish was 10^{-8} M, when the lateral region of the olfactory bulb responded to taurocholate, but the highest threshold was over 10^{-4} M, when the medial region of the olfactory bulb responded to taurocholate. During week 8, the lowest response threshold in the fish was 10^{-8} M, when the lateral region of the olfactory bulb responded to L-alanine, but the highest threshold was 10^{-6} M, when the medial region of the olfactory bulb responded to taurocholate. Response thresholds in other exposed fish tested during weeks 1, 2, 3, 4, 5,7, and 9 varied from 10^{-7} M to concentrations higher than 10^{-4} M.

Weekly Changes in Olfactory Activity

Olfactory activity was assessed by measuring the magnitude of the bulbar EEG response to L-alanine and taurocholate. Data on olfactory activity in both nonexposed and exposed fish are

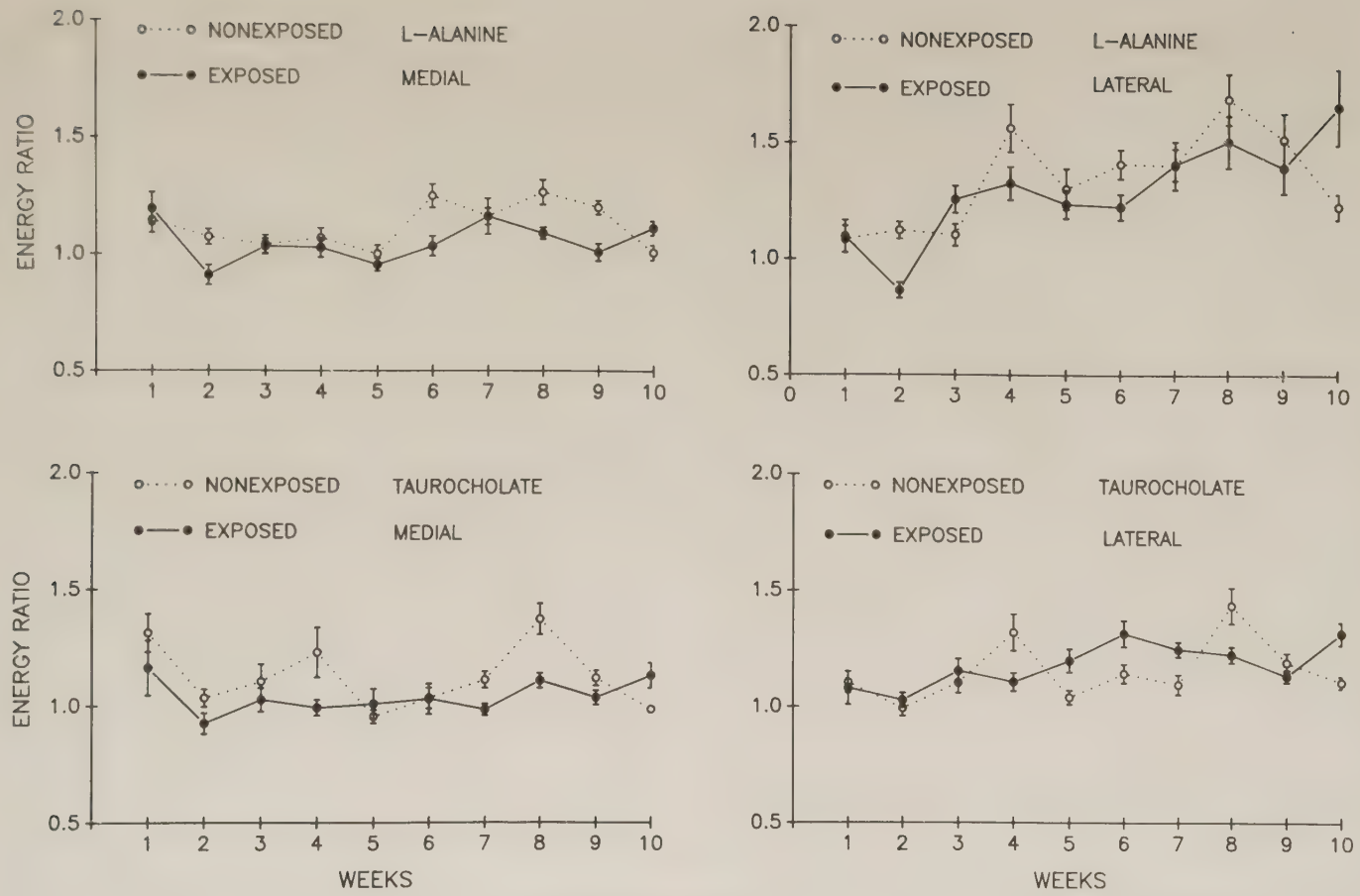

FIG. 2. Mean energy ratio of the EEG response to odor stimulation recorded from the lateral or medial region of the olfactory bulb, per block of five concentrations of L-alanine or taurocholate (10^{-8}–10^{-4} M), in 2-yr-old Atlantic salmon in the course of smoltification. During a 10-wk experimentation period, 10 subgroups of five fish had been exposed to a taurocholate-enriched environment (10^{-7} M) prior to EEG recordings, while 10 other subgroups of five fish had not. The vertical bars represent the mean ± SEM.

expressed in terms of mean energy ratio averaged over five concentrations of a particular odorant (Fig. 2) or calculated for each one of these concentrations (Fig. 3 and 4). In fish either exposed or not exposed to the taurocholate-enriched environment prior to EEG recordings, the energy ratio differed significantly over the 10-wk period when the medial region of the olfactory bulb responded to either L-alanine ($F = 2.63$; df = 9,80; $P < 0.05$) or to taurocholate ($F = 2.10$; df = 9,80; $P < 0.05$).

During most of the 10-wk experimentation period, three different patterns of weekly changes in olfactory activity could be distinguished across all four combinations of recording site and odorant. The first pattern is characterized by two distinct peaks of activity that occurred only in nonexposed fish during week 4 and week 8. This pattern is illustrated in most of the panels in Fig. 2. For the lateral–taurocholate combination, an interaction effect was found for weeks × treatment groups ($F = 2.34$; df = 9,80; $P < 0.05$). This interaction was decomposed to perform an analysis of simple effects between weeks for each treatment group. Overall differences in energy ratio occurred between weeks of nonexposed fish ($F = 3.95$; df = 9,80; $P < 0.01$) but none occurred between weeks of fish exposed to the taurocholate-enriched environment prior to EEG recordings. Comparisons made between consecutive weeks for nonexposed fish revealed significant differences between week 4 and week 5 ($P < 0.05$), week 7 and week 8 ($P < 0.01$), and week 8 and week 9 ($P < 0.05$) (Duncan's multiple range test).

The second pattern of weekly changes in olfactory activity refers to a gradual increase in energy ratio throughout the 10-wk experimentation period. The pattern found for the lateral–alanine combination in both nonexposed and exposed fish is illustrated in the upper right panel of Fig. 3. An interaction was found for weeks × concentrations ($F = 2.84$; df = 36,320; $P < 0.01$). The analysis of simple effects revealed overall significant differences between weeks at 10^{-7}–10^{-4} (F values from 2.75 to 19.09 were all significant at $P < 0.01$) but there were none at 10^{-8} M. Week-to-week increments for a given concentration of L-alanine are illustrated in the upper panels of Fig. 4.

The third pattern displays little or no weekly change in olfactory activity as assessed from energy ratio. This pattern was typically found in exposed fish, as illustrated in most of the panels in Fig. 2. In nonexposed fish, it was only found in the medial–alanine combination.

Prolonged Exposure to a Taurocholate-Enriched Environment

In general, olfactory activity was lower in fish that were exposed to the taurocholate-enriched environment (Fig. 2). For example, the energy ratio in exposed fish was significantly smaller than the energy ratio in nonexposed fish when the medial region of the olfactory bulb responded to L-alanine ($F = 5.58$; df = 1,80; $P < 0.01$) or taurocholate ($F = 4.59$; df = 1,80; $P < 0.01$).

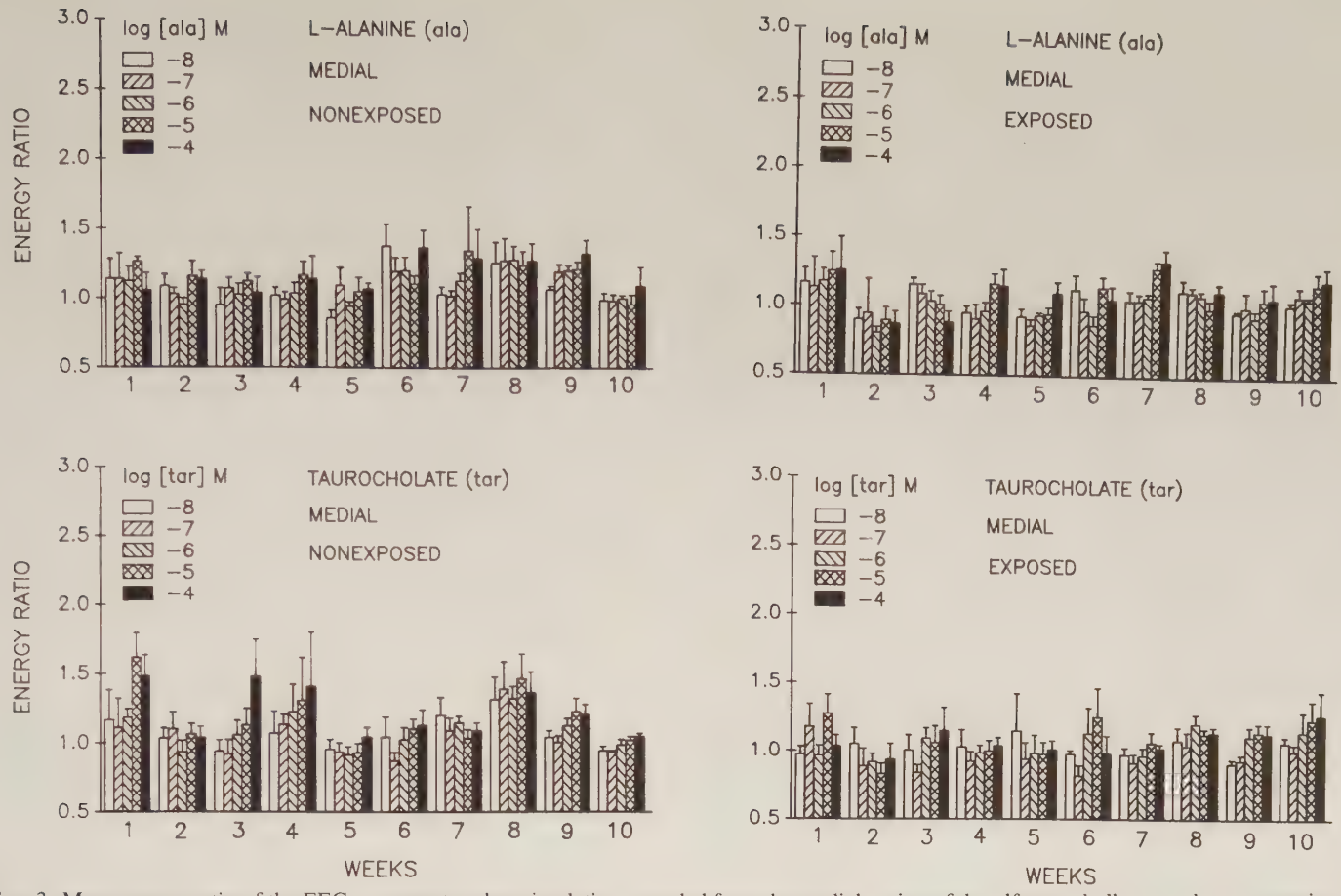

FIG. 3. Mean energy ratio of the EEG response to odor stimulation recorded from the medial region of the olfactory bulb, to each concentration of L-alanine or taurocholate (10^{-8}–10^{-4} M), in 2-yr-old Atlantic salmon in the course of smoltification. During a 10-wk experimentation period, 10 subgroups of five fish had been exposed to a taurocholate-enriched environment (10^{-7} M) prior to EEG recordings, while 10 other subgroups of five fish had not. The vertical bars represent the mean ± SEM.

In the lateral–taurocholate combination, differences between exposed and nonexposed fish were seen only during certain weeks of the 10-wk experimentation period. The energy ratio was significantly smaller in exposed than in nonexposed fish during week 4 ($F = 4.54$; df = 1,80; $P < 0.01$) and week 8 ($F = 4.46$; df = 1,80; $P < 0.01$) but not during any other week of the 10-wk experimentation period.

Activity Increase with Odorant Concentrations

Throughout the 10-wk experimentation period, 10 subgroups of fish (one per week) from the taurocholate-exposed treatment group and 10 subgroups from the nonexposed treatment group were stimulated with five different concentrations of L-alanine and five different concentrations of taurocholate (Fig. 3 and 4). In both exposed and nonexposed fish, there was a general tendency for the energy ratio to increase with increasing odorant concentrations. For example, the energy ratio increased significantly with odorant concentrations when the medial region of the olfactory bulb responded to L-alanine ($F = 5.48$; df = 4,320; $P < 0.01$) or to taurocholate ($F = 5.60$; df = 4,320; $P < 0.01$). Such an increase in the energy ratio with increasing odorant concentrations did not occur during week 1, 2, or 3 when the lateral region of the olfactory bulb responded to L-alanine. In this combination of recording site and odorant, however, a significant interaction of weeks × odorant concentrations ($F = 2.84$; df = 36,320; $P < 0.01$) followed by an analysis of simple effects revealed that the energy ratio increases significantly with odorant concentrations during week 4 ($F = 4.03$; df = 4,360; $P < 0.01$) and during every subsequent week of the 10-wk experimentation period (F values varying from 2.41 to 19.54).

In most of the 20 subgroups of fish, olfactory activity did not increase monotonically with odorant concentrations. A monotonic increase in response to increasing odorant concentrations was observed in only two subgroups of nonexposed fish: those tested during week 4 in the lateral–alanine and the medial–taurocholate combinations and those tested during week 8 in the lateral–alanine combination.

Spatial Distribution of the EEG Response

In nonexposed fish stimulated with an odorant at 10^{-4} M, the relative distribution of the EEG response across the four combinations of recording site and odorant changed throughout the 10-wk experimentation period (Fig. 5). According to a univariate interpretation derived from procedure MANOVA in SPSS-PC, significant changes in this distribution of the EEG response occurred from one to the next pair of 2-wk intervals. There was an overall significant difference between pairs of 2 wk as regards the relative distribution of EEG response, but only the lateral-alanine combination contributed to this difference ($F = 3.29$; df = 4,20; $P < 0.05$). In fish tested during week 2, 2 wk before smoltification, the energy ratio of the EEG response was similar for all four combinations of recording site and odorant, varying only from 1.0 to 1.1. This even spatial distribution of the EEG response in the presence of L-alanine and taurocholate changed to an uneven distribution during

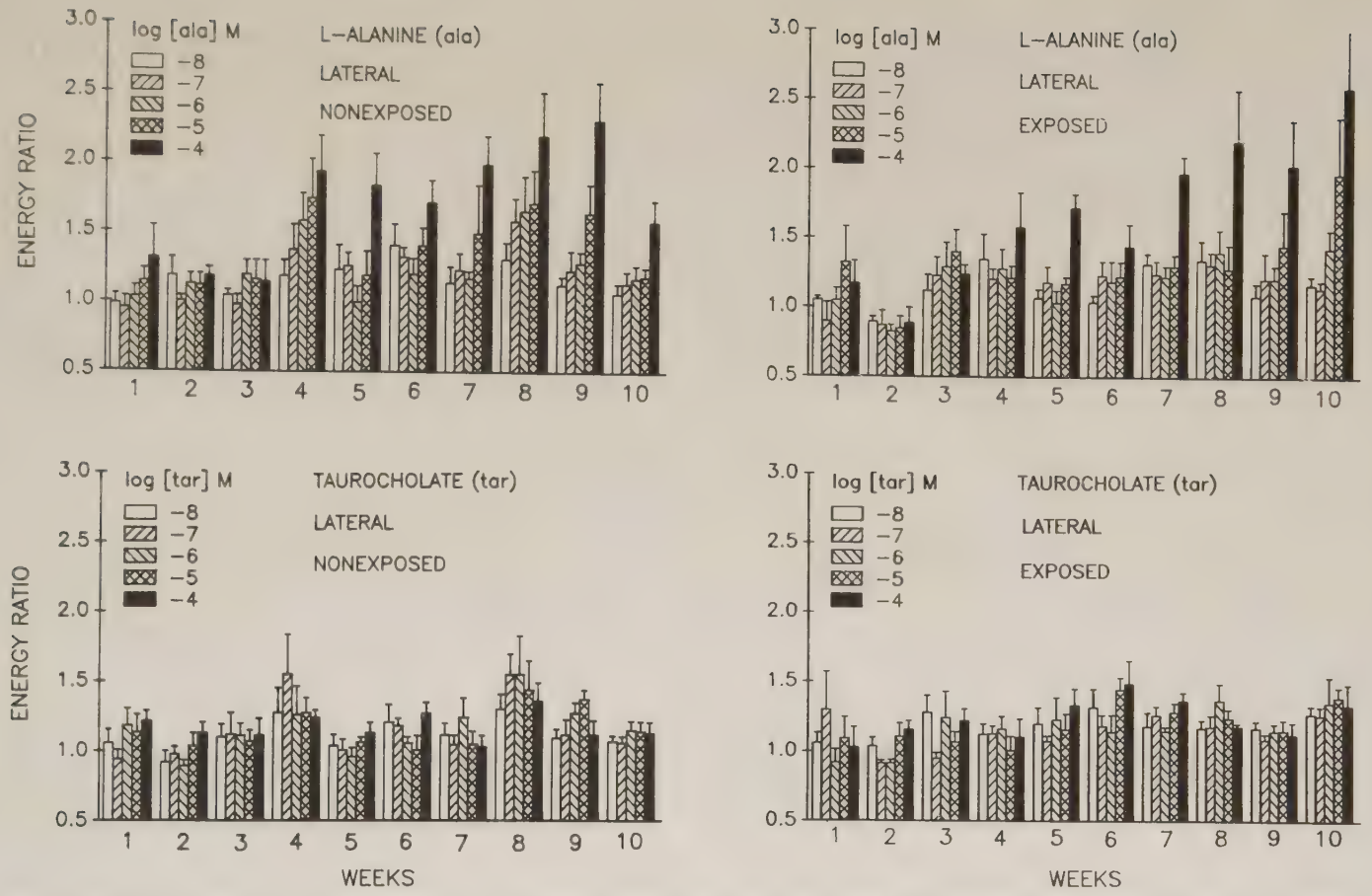

FIG. 4. Mean energy ratio of the EEG response to odor stimulation recorded from the lateral region of the olfactory bulb, to each concentration of L-alanine or taurocholate (10^{-8}–10^{-4} M), in 2-yr-old Atlantic salmon in the course of smoltification. During the 10-wk experimentation period, 10 subgroups of five fish had been exposed to a taurocholate-enriched environment (10^{-7} M) prior to EEG recordings, while 10 other subgroups of five fish had not. The vertical bars represent the mean ± SEM.

week 4 when the first signs of smoltification appeared. As emphasized by subcommand CONTRAST of SPSS-PC, there was a significant difference in the energy ratio of the EEG response between week 2 and week 4 for the lateral–alanine combination ($F = 4.3$, df = 1,20; $P < 0.05$). The uneven distribution of the EEG response dominated by the lateral–alanine combination persisted until the end of the 10-wk experimentation period, but the energy ratio of the EEG response for the lateral–alanine combination during week 10 was smaller than that during week 8 and this difference was significant ($F = 6.4$; df = 1,20; $P < 0.05$).

Discussion

The smoltification of salmon involves a number of physiological, morphological, and behavioral changes (Hoar 1976). We believe that our experiment is the first to document functional changes in the olfactory system, a system which is known to play a key role in migration and homing to the natal river. Firstly, we have demonstrated that olfactory activity, as measured by the responses to L-alanine and taurocholate from the rostrolateral and rostromedial regions of the olfactory bulb, varies in the course of smoltification. The parameters that vary are sensitivity, the dose–response relationship, and the spatial distribution of the responses. Secondly, we have demonstated that exposing fish to taurocholate during smoltification influences olfactory activity. The third interesting observation, and one of specific interest in understanding migration strategies in salmon, is the occurrence of two peaks of olfactory activity that coincide in time with two peaks of odor learning (Morin et al. 1989a, 1989b). Each of these topics is discussed in the following sections.

Changes in Olfactory Activity in the Course of Smoltification

Week-to-week changes

The most important week-to-week changes were found in nonexposed fish when they were tested at two distinct times during the 10-wk period. The first peak of olfactory activity occurred during week 4 when the first signs of parr–smolt transformation were seen. The second peak occurred during week 8, 2 wk after smoltification, when signs of desmoltification first appeared.

Dose–response relationships

The magnitude of the secondary neural responses from the olfactory system of fish, i.e. the induced waves (Adrian 1950) presumably originating from the mitral cell/granular cell interaction (von Baumgarten et al. 1962), increases "proportionately" with the logarithmic increase in concentration of stimulus. This particular dose–response relationship has been reported in salmonids stimulated with L-alanine (Sutterlin and Sutterlin 1971; Belghaug and Døving 1977; Lorentzen 1983; Evans and Hara 1985) and taurocholate (Døving et al. 1980; Hara et al. 1984; Quinn and Hara 1986). The present study

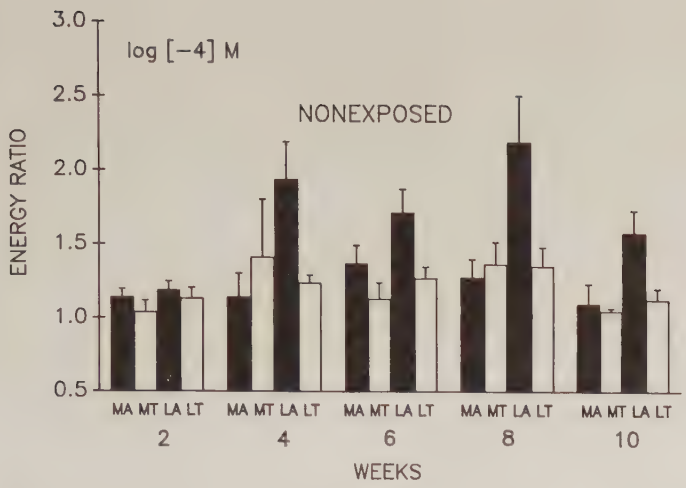

FIG. 5. Mean energy ratio of the EEG response to 10^{-4} M L-alanine or taurocholate, across four combinations of recording site and odorant (MA, medial–alanine; MT, medial–taurocholate; LA, lateral–alanine; LT, lateral–taurocholate), in 2-yr-old Atlantic salmon in the course of smoltification. These five subgroups of five fish tested every 2 wk had not been exposed to taurocholate prior to EEG recordings. The vertical bars represent the mean ± SEM.

demonstrated that such a relationship between magnitude of response and concentration of stimulus is the exception rather than the rule in juvenile Atlantic salmon. The relationship may change dramatically in the course of smoltification. On the other hand, our results agree to some extent with those of Døving et al. (1980) who reported that the response increases proportionally with the log of the concentration of taurocholate, from 10^{-8} to 10^{-4} M, when EEG recordings are made in the rostromedial region of the olfactory bulb but not in the rostrolateral region. We have shown that the EEG response in the rostromedial region of the bulb increases with taurocholate concentration in nonexposed fish tested during week 4 and not in any other period. No such reponse was seen from the rostrolateral olfactory bulb in any time period.

A monotonic increase of the EEG response with concentration of stimulus was observed only in nonexposed fish when the lateral region of the olfactory bulb responded to L-alanine during weeks 4 and 8 and when the medial region of the bulb responded to taurocholate during week 4. Thus, this relationship was typically associated with the two peaks in olfactory activity that occurred in the course of smoltification. This observation suggests that this relationship may be associated in some way with odor learning.

Spatial distribution of the EEG response

In fish not exposed to a taurocholate-enriched environment prior to EEG recordings the relative distribution of the EEG response across the four combinations of recording site and odorant changed drastically throughout the 10-wk experimentation period. Before smoltification, this distribution was not related to the type of odorant used for stimulation. The lateral and medial regions of the olfactory bulb responded similarly to L-alanine; as well, the medial and lateral regions of the bulb responded similarly to taurocholate.

During and after smoltification, our results agree with those of Thommesen (1978) and Døving et al. (1980) when we look at the relative distribution of the EEG response in the lateral–alanine as compared with the medial–alanine combination. We found that the EEG response to L-alanine during weeks 4, 6, 8, and 10 was usually stronger in the lateral rather than the medial region of the olfactory bulb. In adult brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*), Thommesen (1978) reported greater surface potentials in response to amino acids in the lateral as compared with the medial region of the bulb. In studies on Arctic char (*Salvelinus alpinus*) and Arctic grayling (*Thymallus thymallus*), Døving et al. (1980) reported similar results when amino acids were used as odorants.

Our results disagree with those of Døving et al. (1980) when we look at the relative distribution of the EEG response in the medial–taurocholate as compared with the lateral–taurocholate combination. The EEG response to taurocholate was never stronger in the medial than in the lateral region. Before, during, and after smoltification, the response to taurocholate was roughly equal in each region of the olfactory bulb. Døving et al. (1980) reported strong EEG responses in the medial region of the olfactory bulb in response to stimulation with bile salts, including taurocholate, but hardly any response in the lateral region.

Causes of changes in sensitivity

There are about 1000 times more primary receptor cells than secondary neurons in the olfactory epithelium of fish (Gemne and Døving 1969), greatly increasing the probability of responses in the relay cells of the olfactory bulb (van Drongelen et al. 1978). In the frog *Rana ridibunda*, the convergence ratio is functionally involved in the amplification of primary olfactory signals (Duchamp-Viret et al. 1989). In the teleost *Clarias gariepinus*, the convergence ratio between the ampullary electroreceptors and the afferent neurones increases during ontogeny concomitant with an increase in sensitivity (Peters and van Ieperen 1989).

Changes in the convergence ratio of the olfactory system may be caused by an increase in the number of receptors and/or by the branching of receptor cell axons. An increased number of receptor cells could have caused the increase in odor sensitivity that we observed in nonexposed fish tested during weeks 4 and 8 compared with the fish tested before or at any other time during smoltification. Bertmar (1983), who studied the olfactory epithelium of Atlantic salmon, reported twice as many primary lamellae in smolts than in parr. This increase in the surface area of the epithelium may be related to an increase in the number of primary receptor cells during smoltification.

Changes in olfactory sensitivity during week 4, 3 wk after the induction of smoltification, could also be related to the degree of branching of receptor cell axons, which may be a result of greater thyroid activity, also known to occur 3 wk after the induction of smoltification (Morin et al. 1989b). Under such conditions, the convergence ratio will increase, thereby securing the probability of responses in the secondary neurones. Døving (1986) suggested that such changes in the olfactory bulb could lead to increased sensitivity to an odor.

Fish Exposed to a Taurocholate-Enriched Environment

No peaks in olfactory activity

Some of the changes in olfactory activity that occurred throughout the 10-wk experimentation period can be attributed to the effect of prolonged exposure to a taurocholate-enriched environment. When olfactory activity reached a peak in nonexposed fish during weeks 4 and 8, olfactory activity in exposed fish did not increase, suggesting that the prolonged exposure prevented an increase in olfactory activity in the exposed fish. Thus, contrary to what we had expected, the EEG response to odorants in exposed fish did not increase during smoltification. Hara and Brown (1979) did not find any increase in bulbar EEG activity after rainbow trout (*Oncorhynchus mykiss*) had been

exposed to morpholine in the course of smoltification. Unfortunately, they did not compare the EEG response of the olfactory bulb at different times during smoltification. Nevertheless, Morin et al. (1989a) found significant unconditioned reduction of heart rate in response to L-cysteine in smolts exposed to that odorant during a specific time during smoltification. Their findings suggest that odor information is processed in the neural circuitry of the olfactory system as a result of odor learning during a SPOI.

Cause of odor preexposure effects on olfactory activity

A significantly weaker EEG response in exposed than in nonexposed fish during weeks 4 and 8 indicates that exposure to the taurocholate-enriched environment inhibited the neural response measured in the olfactory bulb. One explanation of this phenomenon may be linked to the presence of inhibitory neurons in the olfactory bulb (e.g. granule cells) whose firing rates may have increased under the influence of prolonged exposure to an odor. The results of Wilson et el. (1985), who exposed neonatal rats to pepermint-scented air prior to making single unit recordings from the olfactory bulb in response to peppermint stimulation, are consistant with this idea. They reported significantly more inhibitory and fewer excitatory responses from the mitral cells of exposed rats than from the mitral cells of nonexposed rats. In fish, results derived from field potential studies in which potentials are recorded in different areas of the olfactory bulb following electrical stimulation of the major afferent and efferent pathways support the existence of inhibitory actions of interneurons upon the activity of relay cells (MacLeod 1976; MacLeod and Lowe 1976).

Spatial distribution of the EEG response

The relative distribution of the EEG response across the four combinations of recording site and odorant was similar to that in nonexposed fish, suggesting that prolonged exposure to a single odor before testing had no effect on the spatial distribution of the EEG response to the different odorants used as stimulants. The only exceptions were fish tested during week 6 that exhibited a stronger EEG response to both odorants in the lateral region of the olfactory bulb.

Activity Peaks of Different Sensitivities

Since the first peak of olfactory activity found in this study coincides with a period already associated with a high capacity for odor learning and memory in Atlantic salmon (Morin et al. 1989a), we suggest that this peak is a neural manifestation of the SPOI for this species. During this first peak, the fish were particularly sensitive to both L-alanine and taurocholate, with most response thresholds at 10^{-7} or 10^{-8} M.

The second peak in olfactory activity found in nonexposed fish coincides with an interval of high capacity for odor learning but low capacity for odor memory, as described earlier by Morin et al. (1989a). Therefore, we propose that the second peak in olfactory activity is not related to the SPOI, although we do not rule out the possibility that some imprinting may take place during the second interval. A more limited capacity for imprinting during this second peak could be explained by the lower sensitivity to odorants. Consistent with this explanation, we observed that the second peak of olfactory activity was generally concomitant with higher response thresholds to L-alanine or taurocholate as opposed to the first peak, although both peaks in olfactory activity were of similar magnitude.

Concluding Remarks

In wild populations of anadromous salmonids, smoltification occurs during springtime under a regular increase in daylength and an irregular increase in water temperature. In the present study, we abruptly increased daylength and water temperature to induce smoltification in salmon and to facilitate observations involving changes in physiology. This standardized procedure revealed interesting changes in olfactory functions that can be correlated in time with hormonal changes and with the existence of a SPOI.

How do findings from studies such as ours relate to the migration and homing behavior of wild populations of salmon? This question can be addressed in several different ways. For example, it is known that seaward migration is synchronous in wild populations in spite of the regular change in light and the irregular changes in temperature. It would be interesting to know the underlying physiological and/or other mechanisms that ensure this synchrony in wild populations. Another way to approach the problem would be to examine the behavior of wild salmon smolts during the first and second peaks in olfactory activity, at weeks 4 and 8, respectively, in our terms. It would also be useful to identify the substances, probably of salmon origin, that the salmon "learn" and "memorize". Answers to any of these questions would increase our understanding of the process of salmonid migration.

Acknowledgments

The research was supported by the Norwegian Research Council for Science and the Humanities (NAVF-Norway) and the Directorate for Nature Management (DNF-Norway). The first author was the recipient of three postdoctoral fellowships: (1) Fonds pour la Formation de Chercheurs et l'Aide à la Recherche (FCAR-Québec), Canada, (2) NAVF-Norway, and (3) Olin Fellowship, Canada (Atlantic Salmon Federation). We thank Dr. Hans Nordeng and Dr. Georg Thommesen (Norway) and Dr. Julian J. Dodson (Canada) for reading and criticizing earlier versions of the manuscript. We also thank Dr. Simon Courtenay for making valuable suggestions.

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Trophic Linkage between Stream Centrarchids and Their Crayfish Prey

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Energetic links between smallmouth bass (*Micropterus dolomieu*) and rock bass (*Ambloplites rupestris*) and their crayfish foods were examined in an Ozark stream. A trophic level energy budget was developed by enumerating food habits for different age (size) fish, estimating annual production for both fish and crayfish, and using laboratory- and literature-derived bioenergetic and gross efficiency data. Both fishes began life feeding on small invertebrates (mayflies and chironomids) but within 3 mo switched to a diet of mainly crayfish and Cyprinidae. Total annual production of smallmouth bass was 0.262 g dry weight·m^{-2}·yr^{-1} (6344 J) and rock bass 0.148 g·m^{-2}·yr^{-1} (3607 J). Total annual production of crayfish was 4.15 g dry weight·m^{-2}·yr^{-1} (55 736 J) for *Orconectes luteus* and 5.05 g·m^{-2}·yr^{-1} (62 394 J) for *O. punctimanus*. Only about half of the crayfish production was available to fish, due to size-selective predation and behavioral traits of the prey. A predator–prey model suggested that nearly one third of total crayfish production during their vulnerable period was lost to centrarchids, and that half of the existing biomass was consumed. Fish are probably the major cause of mortality in crayfish and undoubtedly influence crayfish population dynamics and energy flow through the river system.

Dans un cours d'eau des monts Ozark, on a examiné les liens énergétiques entre l'achigan à petite bouche (*Micropterus dolomieu*), le crapet de roche (*Ambloplites rupestris*) et les espèces d'écrevisse dont ces deux poissons se nourrissent. À cet effet, on a mis au point un bilan énergétique au niveau trophique en énumérant les habitudes alimentaires des poissons de diverses tailles, en estimant la production annuelle de poissons et d'écrevisses et en utilisant des données sur la bioénergétique et le rendement brut recueillies en laboratoire et relevées dans des ouvrages publiés. Au début de leur cycle biologique, les deux espèces de poissons se sont nourries d'invertébrés de petite taille, dont des éphémères et des chironomidés pour ensuite, en deça de 3 mo, se nourrir en grande partie d'écrevisses et de Cyprinidés. La production annuelle totale (en poids sec) d'achigan à petite bouche et de crapet de roche s'élevait à 0,262 g·m^{-2}·an^{-1} (6 344 J) et 0,148 g·m^{-2}·an^{-1} (3 607 J), respectivement. Pour ce qui est des écrevisses, la production annuelle totale (en poids sec) atteignait 4,15 g·m^{-2}·an^{-1} (55 736 J) dans le cas de *Orconectes luteus* et 5,05 g·m^{-2}·an^{-1} (62 394 J) dans le cas de *O. punctimanus*. Seule environ la moitié de la production en écrevisses était à la disposition des poissons à cause de la prédation sélective par taille et des traits de comportement des proies. Un modèle prédateur–proie porte à croire qu'environ un tiers de la production totale en écrevisses était la proie de centrarchidés pendant la période vulnérable de leur cycle biologique, et que la moitié de la biomasse présente était consommée. Les poissons sont probablement la principale cause de la mortalité des écrevisses et il n'existe pas de doute qu'ils influent sur la dynamique de leurs populations et le flux énergétique dans le système fluvial.

Received October 11, 1990
Accepted February 7, 1992
(JA754)

Reçu le 11 octobre 1990
Accepté le 7 février 1992

Crayfish and Centrarchidae commonly occur together in warmwater streams and rivers of North America. Their association as predator and prey has long been recognized by anglers and confirmed by numerous food-habit studies (Pflieger 1975). Yet the ecological importance of this relation has rarely been investigated further. Understanding the significance of particular prey depends on quantifying the trophic interaction among species of interest and their major food resource (Waters 1988). Such information should be useful for explaining population and community dynamics and can lead to rational mechanistic-based management of stream productivity. However, the expectations for production-based management of streams have not materialized. Allen's (1951) classic study on the Horokiwi stream in New Zealand concluded that secondary production of benthic invertebrate prey was insufficient to account for trout production. The resulting "Allen's Paradox" (Hynes 1970) has consumed considerable efforts of stream biologists (e.g. Horton 1961; Mann 1965; Allan 1982; Reice 1983) and has continued to raise questions about our methodology and concepts (see review by Waters 1988). Recently some advances have been made in our understanding of trophic relations in regard to the underestimation of invertebrate turnover times (Benke 1984), the role of alternative foods (Hunt 1975), the importance of spatial habitat heterogeneity (Benke 1984; Bowlby and Roff 1986), and the interplay of food with habitat (Mason 1976; Wilzbach 1985). There is evidence accumulating that fish predation controls benthic community structure or production in streams (Bowlby and Roff 1986; Power 1990) and evidence of fish regulation by benthos (Mason 1976; Wilzbach 1985).

The objective of this study was to quantify the trophic relations between crayfish and their principal fish predators, the centrarchids *Micropterus dolomieu* (smallmouth bass) and *Ambloplites rupestris* (rock bass), in a Missouri Ozark stream. I have taken a production-based approach, defining production (sensu Ivlev 1961) as the elaboration of biomass over a period

of time regardless of the fate of that biomass. To accomplish this objective required information on crayfish production, fish production, food habits of fish, behavioral interactions between predator and prey, and some bioenergetic conversions. I used the energy demands for observed fish populations to evaluate the percentage of energy fish derive from crayfish and the percentage of crayfish production passed along to fish and to draw conclusions about the overall ecological importance of the relation.

Species

Orconectes luteus and *O. punctimanus* are common in eastern Ozark Plateau streams of Missouri and Arkansas (Williams 1954) and are the only common species in most of the Ozark National Scenic Riverways (ONSR). Sympatric populations were found to inhabit distinctly different microhabitats (Rabeni 1985). *Orconectes punctimanus* was associated with slow current velocities. Young-of-the-year preferred shallow water with macrophyte cover, while adults were correlated with large substrate particle sizes. *Orconectes luteus* was more evenly distributed among the available microhabitats but tended to concentrate in higher water velocities than did *O. punctimanus*. *Orconectes luteus* densities averaged four times greater than *O. punctimanus* on a streamwide basis.

Smallmouth bass and rock bass are common sympatric species in many Ozark streams (Pflieger 1975). Habitat use in ONSR by both species was highly correlated with some aspect of cover, especially woody structure. Rock bass were usually found near rootwads, while smallmouth bass more often used aggregates of logs and branches (Probst et al. 1984) and tended to concentrate in association with boulders in winter (Todd and Rabeni 1989). Smallmouth bass used higher current velocities than rock bass, and larger individuals of both species used deeper water. Although densities varied considerably with habitat characteristics, rock bass were generally more abundant than smallmouth bass (Covington 1982). Fish condition and growth rates were within the average range for stream populations for both species (Covington et al. 1983).

Study Area

The Current and Jacks Fork rivers in south-central Missouri have mean annual discharges of 41 and 12.5 $m^3 \cdot s^{-1}$, respectively, and are typical of Ozark streams. They are heavily spring fed with a medium gradient ($\sim$1 $m \cdot km^{-1}$) and well-developed riffle and pool sequences. Stream width is typically 5$\sim$25 m, and pools >2 m deep are common. The substrate is predominantly cherty dolomitic limestone, and gravel–cobble particle size is dominant. Periodic spates and overall fast current velocities account for the typical erosional substrate. The deposition of fine materials is limited to side areas, backwaters, and deep pools. On stream margins are frequent beds of water willow (*Justicia americana*), a characteristic emergent plant of the Ozarks that grows in colonies and is especially common around shallow running water in the vicinity of riffles.

Methods

Crayfish Collections

Crayfish were collected at seven locations on the Current and Jacks Fork rivers. Ten replicate samples at each site were taken at five approximately equally spaced intervals each year from May to November 1981 and from April to October 1982. The crayfish species in these streams became essentially inactive at temperatures below 15°C and the sampling periods covered most of the growing season.

Collections were made with a specially designed sampler described in Rabeni (1985). Briefly, a 1-m^2 metal frame with four 40-cm-long metal legs was attached to a fine minnow seine with a lead line on the bottom which covered the front and sides of the sampler, while the downstream end had a tapered 50-cm-long bag. The device worked similarly to a Surber square foot sampler, except we were able to climb into this device to overturn rocks and conduct a complete search of the bottom area. The numbers of samples taken at a site were allotted in approximate proportion to the abundance of habitat type as distinguished by current velocities, substrate particle size, and vegetation, which were in practice selected as riffle, run, pool, and vegetated areas.

Fish Collections

Detailed methods of fish collection are given in McClendon and Rabeni (1987). In summary, fish for production estimates were collected in 1982 and 1983 with a boat-mounted DC electrofishing unit on the Jacks Fork River at a total of 20 different areas ranging in size from 0.25 to 1.31 ha. All fish were weighed, measured, marked by clipping a pectoral fin, and released. Scale samples were taken for age analyses from five fish in every 10-mm length group whenever possible. Each site was resampled about 7 d later to gather density estimates using Chapman's modification of the Petersen formula (Ricker 1975).

Fish for feeding analyses were also collected by electrofishing; two areas on each river were sampled shortly after dark at about monthly intervals from March to November 1980 (Probst et al. 1984). Stomach tubes (Van Den Avyle and Roussel 1980) were used to recover consumed prey from 232 smallmouth bass 100–425 mm in length and 211 rock bass 100–314 mm in length. Food consumption by smaller fish, generally young of the year, was studied by examining 151 smallmouth bass and 71 rock bass caught by trapping or seining in 1985 and 1986 (Livingstone 1987).

Fish Age and Growth

Plastic impressions of scales were interpreted and measurements made to each annulus on an Eberbach scale projector at a magnification of 40×. Correlation coefficients and geometric mean regression formulae (Ricker 1975) were computed for the scale radius – total length relations. Least-squares regression formulae were calculated with the standard logarithmic method for weight–length relations for each fish. Total lengths at annulus formation were calculated using the method of Hile (1941), assuming a linear scale radius – total length relation. Weight at annulus was computed by substituting the calculated total length at annulus into the appropriate weight formula where lengths are in millimetres and weights are wet weights: smallmouth bass, log weight = $-5.2 + 3.12$ log length ($r^2 = 0.99$) (Covington et al. 1983); rock bass, log weight = $-5.13 + 3.20$ log length (Marteney 1983). A dry to wet weight ratio of 1:3 was assumed (Vannote and Ball 1972; Brett and Groves 1979) and used for biomass and production estimates.

Densities and biomasses of the various age groups were estimated by calculating the percentage of the population of each site occurring in each 10-mm size group and multiplying the estimated density by that percentage. Electrofishing bias against smaller individuals was compensated for by the appropriate

correction factor developed for fish in this stream by a comparative electrofishing and underwater observation study (McClendon and Rabeni 1988). The resulting density was multiplied by the mean weight of individuals in the appropriate size group. A summation of all size groups gave an estimate of biomass for each 10-mm size group. The estimates were summed within the appropriate age class to give an estimate of biomass for the entire population.

Crayfish Age and Growth

Orconectes spp. eggs hatch in May or June. A collection in early summer will obtain crayfish at ages of about 0.0, 1.0, and 2.0 yr, while a collection in late fall will obtain crayfish at ages of about 0.5, 1.5, and 2.5 yr. Few crayfish of either species lived beyond 2.5 yr, and those that did contributed little to total production and nothing to the diets of the centrarchids Age composition for crayfish is often determined from length–frequency distributions (Momot 1967; Flint 1975; Price and Payne 1984). Some investigators follow statistical methods, while others use less formal curve-fitting approaches. I chose the latter and patterned the analysis after Momot (1967). Age 0 crayfish are easily distinguished (Rabeni 1985) and their bell-shaped distribution can be followed through time. Where obvious peaks in the older age classes were not evident for particular time periods, I used the average second year's growth which I determined from samples that did have obvious second year peaks. This was about 50% for *O. luteus*, but more variable for *O. punctimanus*. I then constructed somewhat symmetrical normal curves around the means. Where the age classes overlapped, I assigned half the individuals to each age class. This only proved problematical with older age 1 crayfish. However, an error distinguishing older age 1 from young age 2 will have a small effect on estimating total production because production of age 2 individuals accounts for only about 6% of the total.

Dry weights for production estimates were developed from length – dry weight regressions for each sex of each species: *O. luteus* males, log weight = 3.83 log carapace length − 5.05 (r^2 = 0.93); *O. luteus* females, log weight = 3.38 log carapace length − 4.6 (r^2 = 0.93); *O. punctimanus* males, log weight = 3.44 log carapace length − 4.7 (r^2 = 0.96); *O. punctimanus* females, log weight = 3.44 log carapace length − 4.76 (r^2 = 0.93).

Production Estimation

Annual production of fish and crayfish was calculated both by Allen graphs (Allen 1951) and the instantaneous growth method (Ricker 1975). Essentially identical results were obtained (±10%) (C. F. Rabeni, unpubl. data), and use of either method in the paper is for ease of understanding and interpretation.

Fish production is presented as the sum of products of the average biomass and instantaneous rate of growth for each age class (Ricker 1975). Density estimates for each age group for production estimates were obtained from sampling a multiaged population rather than following a single cohort through all years of its life. Such an approach assumes a steady-state condition (recruitment and mortality) over the last several years, and also within years. We made this assumption based on size–frequency analysis of these species from this and other studies in the same stream which showed catch curves typical of populations without weak or missing year classes (Covington et al. 1983; National Park Service, unpubl. data).

Crayfish production was estimated graphically by plotting mean densities and average individual weights for different size (age) groups from collections over 2 yr and by integrating the resulting curve (Allen 1951). While Allen curves are generally expressed using a single cohort, where densities and mean weight are followed from birth to the last survivor, a composite curve can be constructed with information from several age groups in a single population. In this case, cohort production equals annual production.

Energetic Values

Fish feeding

Prey items taken from fish stomachs were weighed and then adjusted for their energetic content to develop an importance index that depicts the usable energetic contribution of each prey item, taking into account the fact that the total energy of a food item is not metabolized by fish (e.g. the chitinous exoskeleton of crayfish). (See Probst et al. (1984) for computational details.) For *Orconectes*, I used 2950 cal·g dry weight^{-1} (12 331 J·g^{-1}) (mean values of King and Ball 1967; Kelso 1973) which was reduced 17% to account for energy lost in feces, which is a mean value for fish feeding on invertebrates with a hard exoskeleton (Brett and Groves 1979). For invertebrates other than crayfish, I used 5028 cal·g^{-1} (21 017 J·g^{-1}) (King and Ball 1967), also reduced by 17% (Brett and Groves 1979). A mean value for Cyprinidae of 5800 cal·g^{-1} (24 244 J·g^{-1}) (Niimi and Beamish 1974) was reduced by 6% to account for the unassimilated fractions (Brett and Groves 1979).

Production energetics

Smallmouth bass and rock bass were assigned a value of 5800 cal·g dry weight^{-1} (24 244 J·g^{-1}), an average of values given for largemouth bass (*Micropterus salmoides*) by Adams et al. (1982). Crayfish were assigned the same value as above (2950 cal·g^{-1}, 12 331 J·g^{-1}) without the 17% reduction.

Results

Fish Feeding

Young-of-the-year smallmouth bass ate very few crayfish, but consumption as a percentage of total usable energy increased as the fish grew, and adult fish obtained over 60% of their energy from crayfish (Fig. 1). A similar pattern existed for rock bass, where consumption of crayfish began when fish were about 25 mm long and increased with fish size so that adult rock bass obtained approximately 70% of their total energy from crayfish. One crayfish species was not preyed upon more than the other, and both fish species fed on crayfish in approximate proportion to the relative abundance of the two species of crayfish (Probst et al. 1984).

Fish ranked second in importance as prey for both species and were almost exclusively Cyprinidae and mainly *Campostoma* spp. Other common food items were invertebrates other than crayfish, mainly insects. Chironomidae and Ephemeroptera were especially important to very small rock bass, those 25–50 mm long.

Fish Biomass and Production

Information was obtained from 645 smallmouth bass. Densities and biomasses varied greatly among sites (41–278 fish·ha^{-1} and 7.9–51.7 kg·ha^{-1}) depending on habitat conditions (McClendon and Rabeni 1987). The mean percent recap-

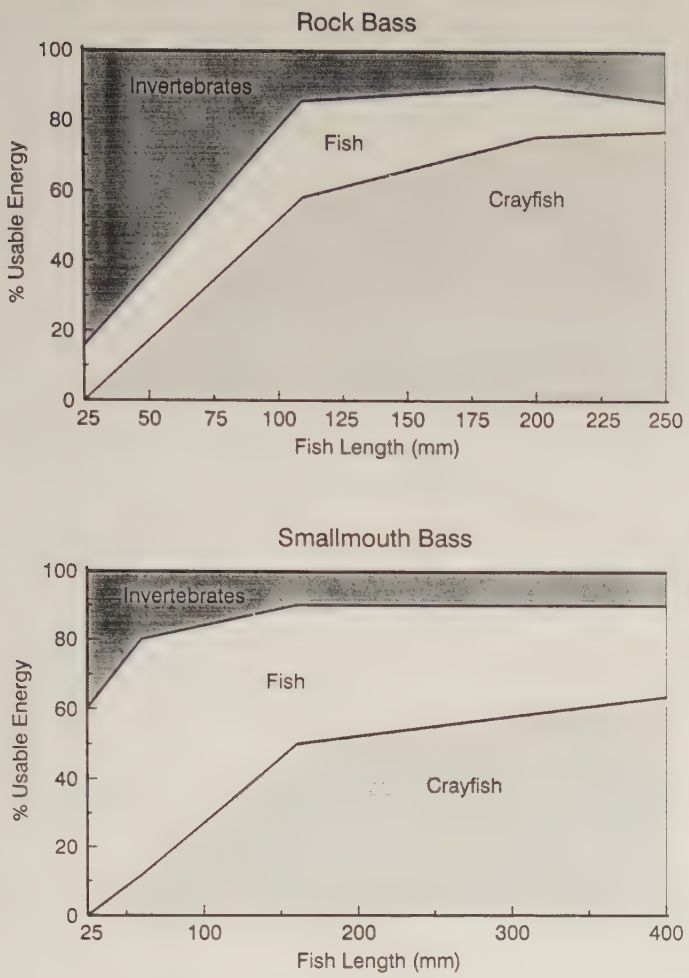

FIG. 1. Comparison of relative importance of three food items in diets of different sizes of smallmouth bass and rock bass from ONSR. Percent usable energy is the percent of total metabolizable calories ingested. Data reanalyzed from Probst et al. (1984) and Livingstone (1987).

ture was 31.5, which ensured adequate estimates of densities. The mean 95% confidence limits of all estimates were within −48 and +98% of the estimates. Mean biomass (age ≥1) was 0.442 g dry weight·m^{-2} (10 715 J) (Table 1), and estimated total annual production was 0.262 g·m^{-2}·y^{-1} (6371 J). The final column of Table 1 represents the production during each age interval that was contributed by crayfish. This information was obtained by multiplying the total production during an age interval by the percentage crayfish contributed to usable energy from Fig. 1. About 57% of total production of smallmouth bass was attributed to energy derived from crayfish.

Densities and biomasses of rock bass also varied greatly among the 20 sites (49–333 fish·ha^{-1} and 6.2–35.3 kg·ha^{-1}). The mean percent recapture was 41 and the 95% confidence limits were all within −42 and +83% of estimated values. Mean biomass of age ≥1 was 0.307 g dry weight·m^{-2} (7442 J), and estimated total annual production was 0.148 g·m^{-2}·yr^{-1} (3607.7 J) (Table 2). The final column of Table 2 represents the production during each age interval that was contributed by crayfish. About 62% of total production of rock bass was attributed to energy derived from crayfish.

Crayfish Production

A contagious spatial distribution was exhibited by both species, and age classes within species, and was correlated with current velocity, substrate particle size, and aquatic vegetation (Rabeni 1985). The *O. luteus* density estimates from the 60 samples per sampling date produced standard deviations averaging 136% (young-of-the-year) and 117% (adult) of estimated densities. Standard errors averaged 42% (young-of-the-year) and 35% (adults) of estimated densities. Such values indicated that approximately 24 samples for young-of-the-year and 48 samples for adults were needed to be 95% confident of being within 20% of the true mean (Elliott 1977). Estimates for *O. punctimanus*, because of lower densities and more selective habitat use, were less precise. The standard deviations, as percentages of the mean, averaged 204 for young-of-the-year and 206 for adults. Standard errors averaged 63% (young-of-the-year) and 64% (adult) of the density estimate. These values indicate that 86 young-of-the-year samples and 109 adult samples would be needed to be 95% confident of being within 20% of the true mean.

Orconectes luteus produced an average biomass of 4.51 g dry weight·m^{-2}·yr^{-1} (55 736 J) (total area under curve, Fig. 2). Production was more a function of survival than of growth rate. The first year of life accounted for a mean of 54% of total production and the second and third years 38 and 8%, respectively. *Orconectes punctimanus* produced an estimated 5.05 g·m^{-2}·yr^{-1} (62 394 J) (Fig. 2). The first year of life of this species accounted for about 62% of total production, with the second and third years accounting for 32 and 6%, respectively.

Production estimates for the two species were similar even though there were differences in densities. *Orconectes luteus* averaged four to five times more individuals, yet it produced about 0.54 g·m^{-2}·yr^{-1} (6661 J) or just 10% less than *O. punctimanus*. This result was due primarily to faster growth and greater mean size of *O. punctimanus*.

Trophic Linkage

Total annual production of crayfish was about 20 times greater than that of the fish. However, not all crayfish production was available to the centrarchids because of size selectivity by fish or behavioral traits of crayfish. Probst et al. (1984) reported that, in these rivers, 90% of crayfish consumed by smallmouth bass were 24–46 mm long (total length) and 90% of crayfish consumed by rock bass were 30–44 mm long. This length range encompasses crayfish weighing about 0.1–1.0 g (dry weight).

The shaded area on the production graphs of each crayfish species (Fig. 2) represents total production lost to mortality during the vulnerable period when crayfish were in a size category susceptible to fish. It is the decrease in numbers multiplied by the average weight during that time interval (see Benke 1984 for further explanation of the method). This production mortality is from predation and other natural causes and is estimated to be 1.77 g·m^{-2}·yr^{-1} (21 825 J) for *O. luteus* and 1.58 g·m^{-2}·yr^{-1} (19 453 J) for *O. punctimanus* or a total of 3.35 g·m^{-2}·yr^{-1} (41 278 J) for both species.

Total fish production was 0.411 g·m^{-2}·yr^{-1} (9978 J) of which 56% or 0.230 g·m^{-2}·yr^{-1} (2838 J) was contributed by crayfish. However, the total amount of consumed crayfish must include not only energy of growth, but also the inassimilable fractions and metabolic costs. I estimated consumption by assuming a gross conversion efficiency, by weight (growth

TABLE 1. Smallmouth bass annual production (m^{-2}) in ONSR as calculated by the instantaneous growth method ($P = GW$) (Ricker 1975). P_g = production in g dry weight, P_j = production in joules, G = instantaneous growth, % CFD = % crayfish in diet, $PDTC_j$ = production due to crayfish in joules.

Age (yr)	Mean length (mm)	Mean weight (g)	G	Density (no.·10^{-4})	Mean weight (g·10^{-4})	Mean biomass (g·10^{-4})	$P_g \cdot 10^{-4}$	P_j	% CFD	$PDTC_j$
1	82.5	1.4		151.0	211.4					
			1.80	(5.6)[a]		246.3	444.8	1078.3	10	107.8
2	147.0	8.5		33.0	281.2					
			0.89	(18.4)[a]		627.0	556.6	1349.4	50	674.7
3	195.0	20.7		47.0	972.9					
			0.61	(26.5)[a]		813.2	494.0	1197.6	50	598.8
4	237.0	38.0		17.2	653.6					
			0.50			835.1	418.8	1015.3	50	507.7
5	278.0	62.7		16.2	1016.5					
			0.44			834.9	367.9	891.9	65	579.8
6	320.0	97.5		6.7	653.2					
			0.41			532.4	218.6	529.9	65	344.4
7	365.0	147.0		2.8	411.6					
			0.32			266.7	86.1	208.7	65	135.6
8	405.0	203.0		0.6	121.8					
			0.22			136.8	30.1	72.9	65	47.4
9	435.0	253.0		0.6	151.8					
			0.08			130.9	10.9	26.4	65	17.2
10	445.0	275.0		0.4	110.0					
						4423.4	2627.9	6370.4		3013.4

[a]Original value compensated for by appropriate correction factor to eliminate electrofishing bias (McClendon and Rabeni 1988).

TABLE 2. Rock bass annual production (m^{-2}) in ONSR as calculated by the instantaneous growth method ($P = GW$) (Ricker 1975). P_g = production in g dry weight, P_j = production in joules, G = instantaneous growth, % CFD = % crayfish in diet, $PDTC_j$ = production due to crayfish in joules.

Age (yr)	Mean length (mm)	Mean weight (g)	G	Density (no.·10^{-4})	Mean weight (g·10^{-4})	Mean biomass (g·10^{-4})	$P_g \cdot 10^{-4}$	P_j	% CFD	$PDTC_j$
1	38	0.2		0.0	0.0					
			2.07			10.0	20.8	50.4	40	20.2
2	75	2.0		10.0	20.0					
			1.44			252.2	365.0	884.9	40	353.9
3	118	8.5		57.0	494.5					
			0.83	(37.9)[a]		573.7	476.4	1154.9	72	831.5
4	153	19.5		34.5	663.0					
			0.40			669.3	271.4	657.9	72	473.7
5	175	29.2		23.1	675.7					
			0.22			631.8	144.2	349.6	72	251.7
6	187	36.7		16.0	588.0					
			0.22			506.7	116.5	282.4	72	203.4
7	201	46.2		9.2	425.5					
			0.20			29.2	59.8	144.9	72	104.3
8	215	56.7		2.8	158.9					
			0.24			140.9	34.0	82.4	72	59.3
9	234	56.7		1.7	122.8					
						3077.0	1488.1	3607.4		2298.0

[a]Original value compensated for by appropriate correction factor to eliminate electrofishing bias (McClendon and Rabeni 1988).

ingestion), of 22% which is the mean of values from 19 studies of fish feeding on invertebrates summarized by Brett and Groves (1979). Accordingly, consumption of crayfish by fish was about 1.04 $g \cdot m^{-2} \cdot yr^{-1}$ (consumption = (0.411 × 0.56)/0.22). Thus, about one third of production lost to mortality during the vulnerable period of crayfish was due to predation by centrarchids.

The mean biomass of the populations of crayfish when the individuals are in the vulnerable size range is about 0.91 $g \cdot m^{-2}$ for *O. luteus* and 0.66 $g \cdot m^{-2}$ for *O. punctimanus* for a total of 1.57 $g \cdot m^{-2}$ (Fig. 2) for both species. During this time of crayfish vulnerability, fish must consume 1.04 $g \cdot m^{-2}$, or about two thirds of available mean biomass, to realize their estimated production.

Discussion

A production-based analysis as used in this study can help evaluate the strength of predator–prey relations and infer the ecological relevance of fish–crayfish interactions and whether food could become limiting to a fish population (Waters 1988). A shortcoming of this approach relates to the formidable sampling problems in a highly heterogeneous environment associated with complex habitat selection patterns of both fish and

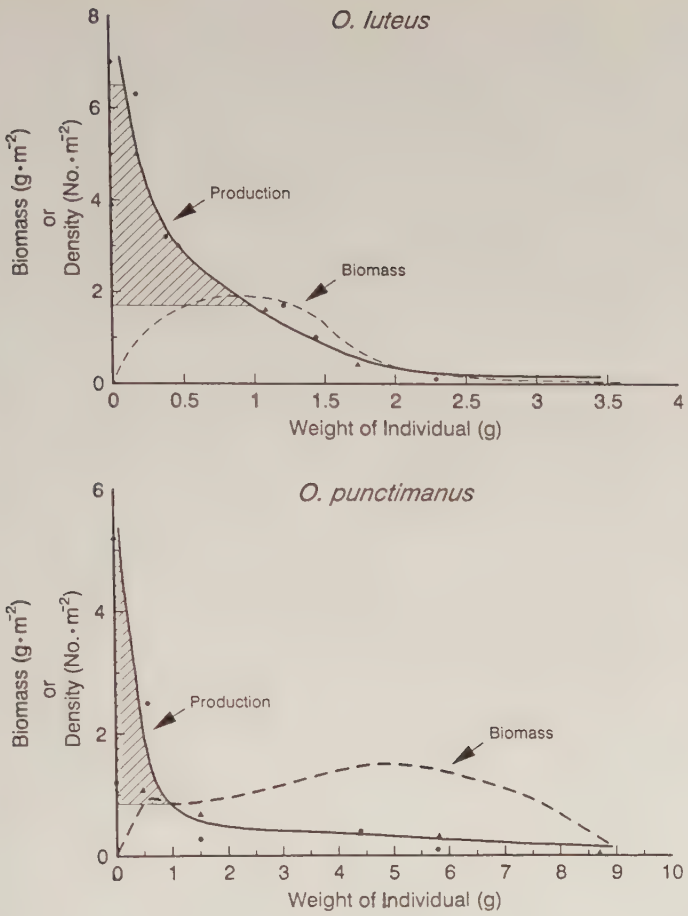

FIG. 2. Production and biomass dry weight graphs for *O. luteus* and *O. punctimanus* from ONSR. The area beneath the solid curves estimates total annual production; shaded areas equal production lost during the interval when crayfish were vulnerable to predation from smallmouth bass and rock bass; triangles = 1981 samples and circles = 1982 samples. Data points for the smallest individuals of both species in 1982 were not included in the curve fitting because densities are considerably lower than those of larger individuals of the same cohort and obviously a sample error. Broken lines represent biomass at age derived from the production graph.

crayfish. Concentrating all sampling in a localized area, say a riffle–pool sequence, would have resulted in a more statistically precise model, but probably one not typical of the stream system. The lack of population-level bioenergetics conversions, especially food conversion functions, also likely affects accuracy of the results. So while the final estimates are somewhat speculative, the conclusions allow for some variations in the assumptions and should be true.

Conclusions of this study are that crayfish are the most energetically important element in the diets of rock bass and smallmouth bass and that predation accounts for a significant percentage of total mortality in crayfish. That predators consumed nearly a third of crayfish production and nearly two thirds of their mean biomass during the period when crayfish were of vulnerable size indicates the potential for control of crayfish population dynamics by centrarchids with a concurrent influence on the well being of the fish populations.

Estimates of secondary production for the species in this study fall within the rather narrow boundaries of published values. The few existing studies indicate a range of 0.2–1.4 g dry weight·m^{-2}·yr^{-1} for rock bass (Pajak and Neves 1987; Mahon et al. 1979) and 0.6–1.8 g·m^{-2}·yr^{-1} for smallmouth bass (summarized by Reed and Rabeni 1989). The production of stream-dwelling populations of crayfish generally ranges from 1.7 to 11.2 g·m^{-2}·yr^{-1} (Vannote and Ball 1972; Mason 1975; Huryn and Wallace 1987). Crayfish production tends to be substantially greater than that of its main predators and could potentially support existing populations of fish.

Quantifications of fish–crayfish interactions have been few. Momot (1967) and Gowing and Momot (1979) studied brook trout (*Salvelinus fontinalis*) predation on the crayfish *O. virilis* in Michigan lakes. They concluded that only large fish ate crayfish, only small crayfish were eaten, and that predation was not an important population control mechanism. The situation is likely different in streams where predators are larger and crayfish less abundant. Roell (1989) modeled smallmouth and rock bass predation on crayfish in the New River, West Virginia, and estimated that fish predation accounted for as much as two thirds of the production of age 1 and 2 crayfish. In the Red Cedar River, Michigan, smallmouth bass consumed an estimated 15% of the annual crayfish production (Vannote and Ball 1972) and speculations were that a large rock bass population would increase total predation to over 50% of crayfish production.

Nonetheless, factors other than total calories are also important in evaluating the strength of the interaction between these two groups, among which are crayfish behavior, fish behavior, and alternative foods.

The rather narrow size range where crayfish are vulnerable to predation amounts to about half of their total production. Very small crayfish are ignored or unavailable. My observations indicate that large crayfish are immune to attack because of their defensive abilities, to the extent where these individuals often remain totally exposed during periods of darkness (also see Stein and Magnuson 1976).

Crayfish would not seem to be a particularly good food source because their usable caloric content per gram is about half that of benthic invertebrates or fish, especially in situations such as the ONSR where abundant alternative food sources appear to be present (i.e. over 60 species of fish). However, crayfish are probably more vulnerable than these alternative prey, at least during certain times of their life cycle. Both fish predators, when young, are found in areas of high prey density (Livingstone and Rabeni 1991). A change in diet by underyearling smallmouth bass, at a size of about 25 mm in ONSR, from predominantly insects to fish may indicate an efficient feeding strategy. This sharing of habitat by small fishes and their invertebrate and fish prey changes as the centrarchids grow. Studies have shown that cyprinids in small Ozark streams are not closely associated with larger black basses (Power et al. 1985; Gorman 1988), a behavior which may make cyprinids more difficult to capture than crayfish.

Production is an integration of biomass, recruitment, and instantaneous rates of growth and mortality. Modeling production from field observation is difficult because of unanticipated responses in one or more parameters when another is changed, and predicting the effects of fish predation on crayfish populations in Ozark streams is impossible without knowledge of crayfish compensatory mechanisms. For example, Momot and Gowing (1977a, 1977b) and Morrissy (1975) suggested a self-regulating mechanism for population numbers of crayfish due to the effects of intrinsic feedback loops that were driven by density-dependent factors and the basic productivity of the system. One may infer from these studies that an increase in predation under the right conditions could actually increase production.

Predation effects may also vary depending on local conditions. Factors known to influence predation rates between fish and benthic invertebrates include heterogeneity of habitat (Wilzbach 1985) and extreme abiotic events (Walde 1986), both of which are common in Ozark streams. The conclusions of this study are based on data from broad temporal and spatial scales and relied on mean conditions. Ozark streams with their highly heterogeneous habitats offer variable suitabilities for the biota. Localized habitat conditions favoring high densities of centrarchids are not necessarily those favoring high densities of crayfish; therefore, some stream sections are potentially strongly structured by predation, while other sections are not.

There is circumstantial evidence of the existence of controlling mechanisms between centrarchids and crayfish. For example, at 11 sites in Ohio streams, Mather (1990) found an inverse relation between densities of crayfish and their fish predators smallmouth bass, rock bass, and creek chub (*Semotilus atromaculatus*). The relation became most pronounced where fish densities were high. In the New River system of Virginia and West Virginia, growth of smallmouth bass was density dependent and possibly related to food resources (Austen and Orth 1985). Fish from river reaches in Virginia had slower growth rates, lower condition, and lower survival than fish from reaches in West Virginia. Virginia smallmouth bass also consumed a significantly greater percentage of insects and less crayfish, and the average volume of ingested crayfish was less in all size classes of fish. Imposition of a 305-mm length limit for anglers on smallmouth bass in a Missouri Ozark stream resulted in increased density of smallmouth bass less than 305 mm in length and a decrease in growth rates (Fajen 1981). There is also evidence to the contrary. For example, intensive harvesting of crayfish from a small West Virginia stream affected neither crayfish densities nor the predatory fishes (Brandt and Schreck 1975).

In a system such as an Ozark stream, it is possible that heavy predation concentrated on medium-sized crayfish transfers substantial energy to the next trophic level, permits better growth of the remaining small crayfish, and assures the existence of an adequate number of large, nonvulnerable crayfish for breeding. Because of heavy predation, centrarchids of ONSR may indirectly affect rates of basic stream processes. Total benthic invertebrate production (excluding crayfish) at two different sites in ONSR was estimated to be 9.0 and 9.9 $g \cdot m^{-2} \cdot yr^{-1}$ (Gossett 1992). Therefore, crayfish may account for nearly half of the total invertebrate production and have the potential to dominate energy flow through the invertebrate assemblage in ONSR as they may in other systems (Momot et al. 1978). Other ecologically important effects of size-selective predation of fish on crayfish include potential for increasing the replacement rate of one species by another (*O. sanborni* by *O. rusticus* in midwestern streams (Mather 1990) and influences on habitat choice by smaller crayfish (Rabeni 1985). The important next step is to build on these first-order estimates to examine response of the prey to different levels of predation pressure. An understanding of the underlying mechanisms involved will most likely require a manipulation of both predator and prey densities under field conditions.

Acknowledgments

This is a contribution from the Missouri Cooperative Fish and Wildlife Research Unit (U.S. Fish and Wildlife Service; Missouri Department of Conservation; The School of Natural Resources, University of Missouri; and Wildlife Management Institute cooperating). D. Galat, R. Hayward, and D. Orth reviewed the manuscript.

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Particulate Organic Carbon Supply and Trophic Dynamics in a Mississippi Flood-Control Reservoir Dominated by Gizzard Shad (*Dorosoma cepedianum*)[1]

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Aumen, N. G., C. L. Crist, D. E. Miller, and K. O. Meals. 1992. Particulate organic carbon supply and trophic dynamics in a Mississippi flood-control reservoir dominated by gizzard shad (*Dorosoma cepedianum*). Can. J. Fish. Aquat. Sci. 49: 1722–1733.

Sources of particulate organic carbon (POC) and trophic–dynamic relationships were studied in a reservoir with low sportfish populations. Fish community structure and POC input from tributaries, phytoplankton primary production, and vascular vegetation on mudflats were estimated. Gizzard shad (*Dorosoma cepedianum*) averaged 39.8% of the total fish biomass in 1988, and as much as 94% of the total forage fish biomass was too large to serve as prey for most predators. Phytoplankton primary productivity averaged 1182 mg $C \cdot m^{-2} \cdot d^{-1}$ in 1987 and 1988, contributed 33.57 Gg $POC \cdot yr^{-1}$ to the reservoir, and apparently was phosphate limited. POC inflow from tributaries contributed 60.00 Gg $\cdot yr^{-1}$ with 79% of POC <75 μm in size. Winter and spring stormflow was responsible for 92% of the total POC transported. Considering POC size fractions available to gizzard shad, POC input from tributaries, phytoplankton, and mudflats contributed 21, 54, and 25% of the total POC input, respectively. The trophic–dynamic analysis indicated that phytoplankton POC was adequate to support the reservoir fish community. A more efficient transfer of carbon in the food web might be accomplished by stocking with a smaller forage fish, such as threadfin shad (*Dorosoma petenense*), which are not common in the reservoir.

On a examiné les sources de carbone organique particulaire (COP) et les relations trophiques–dynamiques dans un réservoir abritant de faibles populations de poissons gibiers. On a aussi effectué des estimations en ce qui concerne la structure de la communauté ichtyenne et l'apport en COP issu de tributaires, la production phytoplanctonique primaire et la densité des plantes vasculaires présentes sur les barres de vase. En moyenne, l'alose à gésier (*Dorosoma cepedianum*) constituait 39,8 % de la biomasse ichtyenne totale en 1988; par contre, jusqu'à 94 % de la biomasse totale de poissons fourrage était de trop grande taille pour servir de proie à la plupart des prédateurs. La productivité phytoplanctonique primaire atteignait en moyenne 1 182 mg $C \cdot m^{-2} \cdot d^{-1}$ en 1987 et 1988, contribuait 33,57 Gg $COP \cdot an^{-1}$ au milieu et était en apparence limitée par la faible teneur en phosphate. L'apport de COP issu de tributaires, dont 79 % était de taille inférieure à 75 μm, s'élevait à 60,00 Gg $\cdot an^{-1}$. Les écoulements d'averse hivernale et printanière étaient responsables du transport de 92 % du COP total. En ce qui concerne les classes granulométriques de COP dont disposaient l'alose à gésier, l'apport de COP venant de tributaires, du phytoplancton et des barres de vase s'élevait respectivement à 21, 54 et 25 % de l'apport total en COP. L'analyse trophique–dynamique révèle que le COP élaboré par le phytoplancton suffisait à nourrir la communauté ichtyenne du réservoir. On pourrait obtenir un transfert plus efficace du carbone dans le réseau alimentaire en empoissonnant le réservoir de poissons fourrage de plus petite taille, comme l'alose à filament (*Dorosoma petenense*), que l'on n'y retrouve pas communément.

Received July 17, 1990
Accepted February 10, 1992
(JA651)

Reçu le 17 juillet 1990
Accepté le 10 fevrier 1992

To improve knowledge of reservoir food webs and to increase our ability to manage fishery resources, the input of particulate organic carbon (POC) and potential effects on the fish community were studied in flood-control reservoir experiencing declining sportfish populations. Three potential sources of POC to Sardis Reservoir, Mississippi, were examined: transport from inflowing tributaries, phytoplankton primary production, and primary production on exposed mudflats (the latter addressed by Aumen et al. 1991). These data were then used to conduct a trophic–dynamic analysis of Sardis Reservoir as described by Adams et al. (1983) to determine what levels of fish production the reservoir is capable of supporting.

A review of creel surveys conducted by the Mississippi Department of Wildlife, Fisheries, and Parks (MDWFP) suggests that except for increases subsequent to major flood events (1973, 1983), catch per unit effort (CPUE) of sport fish is low,

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TABLE 1. Estimated annual sportfishing effort and catch per unit effort (CPUE), Sardis Reservoir.

Year	Average total effort (h)	Average total CPUE ($kg \cdot h^{-1}$)[a]	Reference
1958–66	251 900	0.32	Schultz 1967
1975	322 609	0.84	Schultz et al. 1976
1976	434 672	0.54	Schultz 1978
1977	290 032	0.38	Schultz 1978
1986	881 049	0.35	Thompson 1987
1988	316 395	0.23	Meals and Kiihnl 1989
1989	326 933	0.22	Meals and Kiihnl 1990

[a]Does not include caught-and-released fish.

particularly in 1988 and 1989 (Table 1). In response to this observation, intensive monitoring of the reservoir fish community was initiated by the MDWFP in 1988. To enhance sportfish populations, Adams et al. (1983) suggested that management directed at enhancing lower trophic levels may be the best approach. However, available information on organic matter supply and trophic dynamics in reservoirs is sparse. Only one prior study directly addressed the relative contributions of autochthonous and allochthonous inputs to a reservoir organic matter budget (Lind 1971), even though this partitioning influences higher trophic levels. In a review of reservoir primary production, Kimmel et al. (1990) stated that out of 17 southeastern reservoirs for which both phytoplankton productivity and fish standing crop data are available, at least seven require some input of allochthonous organic materials from the tributaries to sustain the biological community.

Characteristics of individual reservoirs can vary between those of slow-moving rivers and well-flushed lakes and depend primarily on hydraulic and nutrient loadings, basin morphometry, and flushing rates (Kimmel et al. 1990). These characteristics can influence drastically the nature and quantity of organic carbon that is available to the biological community (Taylor 1971; Goldman and Kimmel 1978; Groeger and Kimmel 1984). Biological productivity in reservoirs that have relatively slow flushing rates depends primarily on autochthonous organic matter such as phytoplankton, periphyton, and aquatic vascular plants. Reservoirs that have higher flushing rates may receive a substantial proportion of their organic matter from allochthonous sources in the form of woody debris, leaves, fine particulate organic matter, and dissolved organic matter.

The Yazoo River and its tributaries comprise the major drainage network for northern Mississippi. One of four flood control reservoirs in this region is Sardis Reservoir, which was constructed on the Little Tallahatchie River in 1940 (Fig. 1). Sardis Reservoir varies between a slow-moving river and a well-flushed lake depending on the water elevation, the tributary discharge entering the system, and the rate of outflow through the reservoir outlet structure. The large annual water level fluctuation (approximately 7.4 m) from flood-control operations results in corresponding variation in physical parameters (Table 2). This large, shallow system is an important contributor to the local economy as a recreational site for anglers and boaters. Heavy fishing pressure results from the reservoir's large size, proximity to a large population center (Memphis, Tennessee), and reputation as a good fishing lake, particularly for crappies (*Pomoxis* spp.). In 1986, the greatest ever fishing pressure and fish harvest were recorded, with 311.45 Mg of fish harvested over 881 049 man-hours expended (Table 1). High fishing pressure during years with below-average water levels (1984–88) was followed by dramatically reduced effort and CPUE, particularly in 1989 (Table 1).

High fishing pressure undoubtedly contributes directly to reduced sportfish populations. In 1989, size and creel limits were imposed on black basses (*Micropterus salmoides* and *M. punctulatus*) and crappies (*Pomoxis annularis* and *P. nigromaculatus*) to improve their abundance and size structure (Meals and Kiihnl 1989). However, reduced fishery yields may also be due to reservoir aging and decreases in nutrient inputs, to shifts in competition or predation, or to lack of available prey (Kimmel and Groeger 1986).

Gizzard shad (*Dorosoma cepedianum*) are a primary food source for piscivorous fishes such as largemouth bass and white crappie (Dendy 1946; Storck 1986). Rotenone studies of Sardis Reservoir have shown that gizzard shad dominate fish population biomass (Schultz 1978; Meals and Kiihnl 1989, 1990), which is common in the Southeast (Grinstead et al. 1977). However, length/frequency data show that most shad are too large to be consumed by the majority of predators (Schultz et al. 1976; Garavelli 1981, 1982; Meals and Kiihnl 1989).

To better understand the trophic–dynamic structure of southeastern reservoirs with abundant gizzard shad, and to suggest possible remediation of the sport fishery in Sardis Reservoir, a study was conducted with the following objectives: (1) to determine the relative importance of various sources of POC to the reservoir food web, (2) to investigate factors that may limit reservoir productivity, and (3) to determine if phytoplankton production alone could support the existing reservoir fish production.

Materials and Methods

Meteorological Conditions

Precipitation and reservoir water level at the Sardis Reservoir dam were recorded automatically at 1-h intervals from January 1987 to December 1988 by the U.S. Army Corps of Engineers. Precipitation and reservoir water level were measured using a Handar model 44A Raingage tipping bucket and a Handar model 436A incremental shaft encoder, respectively.

Fish Population Structure

A rotenone survey of Sardis Reservoir was conducted in August 1988 to estimate the reservoir fish population structure. Two coves and one block site (with the shoreline serving as one side of the block) having a total area of 2.23 ha were sampled. Methods closely followed Davies and Shelton (1983), except block nets (mesh size = 1.3 cm) were set before dawn the day of sampling. Fish were sorted according to species, measured into 2.5-cm size groups, and weighed on the first day of collection. Fish were measured but not weighed on the second day of collection. Rotenone data were analyzed using the Fisheries Resources Analysis System (Miranda 1988).

Phytoplankton Primary Production

Sampling stations

Three reservoir sampling stations wre used when the water level was high whereas two (Stations 1 and 2) were used when the water level was low (Fig. 1).

Primary productivity measurements

Gross primary productivity was estimated at approximately 3-wk intervals from March 1987 until January 1989 using an in situ light/dark oxygen bottle method (American Public Health

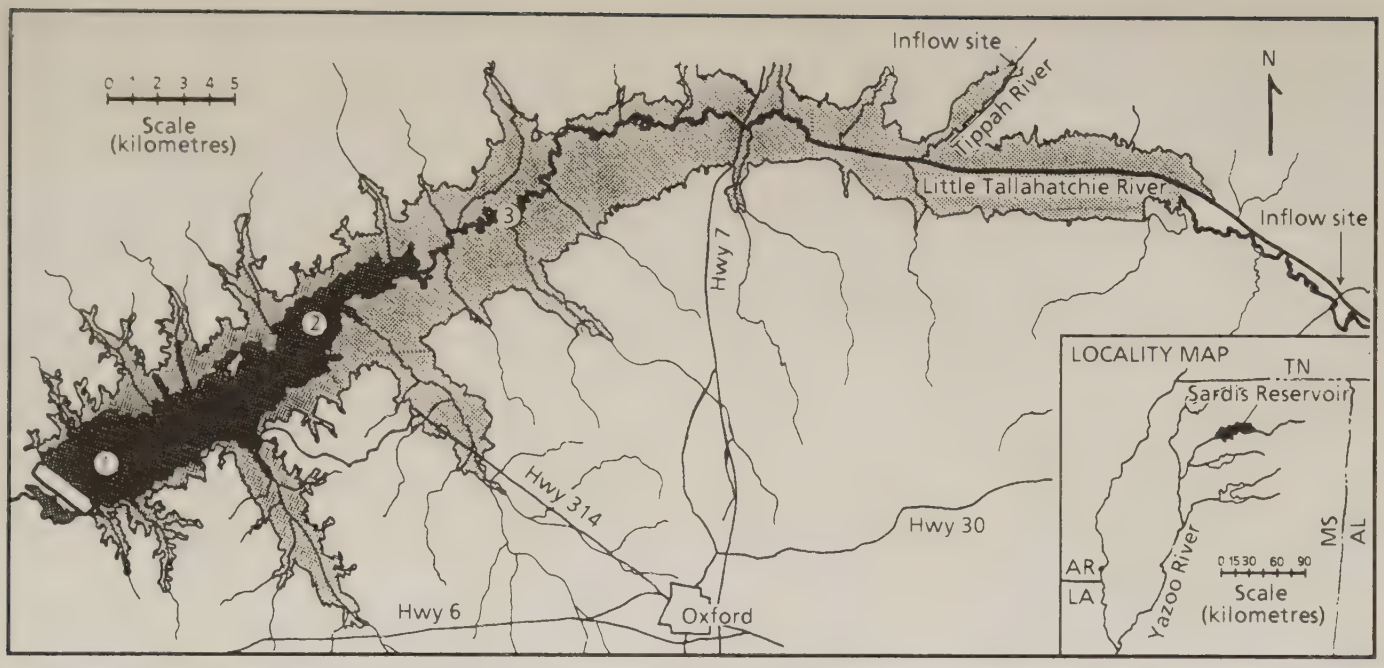

FIG. 1. Map of Sardis Reservoir (latitude 34°24′32″ N, longitude 89°47′56″ W) illustrating sampling locations and drainage area. Stippling indicates water level and surface area extremes; dark stippling indicates conservation pool water level (71.9 m above MSL) and light stippling indicates flood-control pool water level (85.8 m above MSL).

TABLE 2. Physical characteristics of Sardis Reservoir.[a]

Water elevation (above MSL)	
Conservation pool	71.9 m
Recreation pool	79.3 m
Flood-control pool	85.8 m
Water surface area	
Conservation pool	4 452 ha
Recreation pool	12 384 ha
Flood-control pool	23 675 ha
Shoreline length	
Conservation pool	97 km
Flood-control pool	595 km
Shoreline development index	
Conservation pool	4.3
Flood-control pool	10.9
Mean depth	
Conservation pool	2.9 m
Recreation pool	5.6 m
Flood-control pool	8.2 m
Watershed area	4 000 km^2
Water detention time	37–320 d[b]

[a]Source: U.S. Army Corps of Engineers, Vicksburg District, and this study.

[b]Detention time values from U.S. Army Corps of Engineers records for calendar year 1977.

Association et al. 1985; Crist 1988). Water samples wre collected by VanDorn bottle from surface (0.5 m below the surface), middle (halfway between surface and bottom sample depths), and bottom (0.5 m from the bottom) depths, placed in triplicate 300-mL light/dark BOD bottles, and suspended from floating rafts at the depths from which they were collected. Incubations took place over a 4-h period at midday. Light penetration was estimated by determining Secchi disk depths at the time of incubation. Samples were fixed chemically in the field, returned to the labortory, and dissolved oxygen concentrations were determined using a micro-Winkler technique. The dissolved oxygen produced during incubations was converted to the amount of carbon fixed using standard equations (American Public Health Association et al. 1985). Daily carbon fixation was estimated by determining the fraction of the total daily solar radiation that occurred during incubation and applying the appropriate correction value to the data. Two-way ANOVA (Statistical Analysis Systems, Raleigh, NC) was performed to assess the effect of sampling location and depth on primary productivity.

Nutrient enrichment

Enrichment experiments were conducted in April and October 1987 and February and March 1988 by adding various concentrations of phosphate, nitrate, and ammonia (as K_2HPO_4, KNO_3, and NH_4Cl) alone and in combination to standard light/dark bottle incubations described above. At least six replicates of each enrichment and control treatment (containing only background nutrient concentrations) were used. The analyses of phosphate, nitrate, and ammonia were performed according to American Public Health Association et al. (1985) guidelines. Enrichment experiments were conducted at all three incubation depths.

POC Input from Tributaries

Sampling stations

To determine the POC budget for Sardis Reservoir, sampling of both inflow and outflow was conducted approximately once per month during the same time period when primary productivity measurements were made. Because it was not feasible to sample every inflowing tributary to the reservoir, samples were collected from two of the largest tributaries that drained a majority of the reservoir catchment. Their combined drainage areas represented 57% of the total reservoir catchment area, and this percentage was used as a correction factor to determine the total input of POC by extrapolation. For inflow, the sites selected were the Little Tallahatchie River at Highway 30 and the Tippah River approximately 6.5 km upstream from the main stem of the Little Tallahatchie River (Fig. 1). The Little

Tallahatchie River sampling site was located in Union County approximately 32 km north of Oxford on the Highway 30 bridge. This site was chosen because it was accessible safely from the bridge at all river stages and had a continuous stream gauging station linked to an Environmental Data Transmitter satellite network, providing real-time measurements of river stage and rainfall. The Tippah River station was located approximately 8 km from Bethlehem, Mississippi, near Potts Camp and had a similar gauging station and safe access from a bridge (Fig. 1).

The outflow station was located below the dam near the dam outlet structure. The samples were taken as close to the outlet as possible from a boat.

CPOM, FPOM, and VFPOM input measurements

Three size classes of particulate organic matter were selected to measure as part of the reservoir carbon budget. Their designations and size classifications were as follows: coarse particulate organic matter, CPOM (>1 cm); fine particulate organic matter, FPOM (75 μm – 1 cm); and very fine particulate organic matter, VFPOM (0.45–75 μm).

A net (mesh size = 1 cm; 0.56-m^2 opening) was used to capture CPOM. The net frame was equipped with flotation and was deployed from the bridge or boat to capture CPOM in transport. A current velocity meter was attached at the opening to calculate the volume of water sampled. CPOM samples collected in this fashion were dried at 50°C for 24 h, weighed, and ground using a Cycloteck tissue grinder. Approximately 500 mg of each sample was then combusted at 525°C for 1 h, dried to constant weight, and percent organic matter was calculated gravimetrically.

A submersible pump lowered from the bridge or boat to 0.5 m below the water surface was used to collect FPOM and VFPOM. CPOM was excluded from the pump intake by a screen with a 1-cm mesh size. FPOM was retained by pumping water through a sieve with a mesh size of 75 μm and the collected material was washed into a whirlpak bag. VFPOM was sampled by collecting the flow-through from the 75-μm sieve, returning it to the laboratory, and filtering through precombusted Whatman GFC glass-fiber filters with an effective pore size of 0.45 μm. Although it is recognized that particles such as picoplankton smaller than 0.45 μm are present in aquatic ecosystems (Stockner and Anita 1986), this pore size was selected because of its common usage in aquatic ecology (Ward et al. 1990). The glass-fiber filters were then dried for 12 h at 50°C and weighed. Percent organic matter of both FPOM and VFPOM was calculated using the combustion method described above. Carbon–hydrogen–nitrogen content (CHN) of particles collected from June to October 1987 was estimated using a Perkin Elmer model 240B CHN analyzer. CHN analyses were conducted by the laboratory of Dr. W. O. Smith, Jr. (Botany Department, University of Tennessee, Knoxville, TN 37996). Two-way ANOVA and Fisher's least significant difference (LSD) tests (Statistical Analysis Systems, Raleigh, NC) were performed to assess the effect of sample collection site and size fraction on C:N ratios.

Stormflow sampling

To assess the relative contribution of stormflows to the input of POC, three storm events (two during the rainy season, January 1988 and February, 1989, and one in the fall, September 1988) were sampled in the manner described above. For the purpose of this study, a storm event was defined as one causing discharge in the Little Tallahatchie River to increase above 1000 $ft^3 \cdot s^{-1}$. River stages were monitored continuously during periods of high rainfall, and when conditions appeared to satisfy the storm definition, a sampling program was initiated with efforts directed at obtaining samples during baseflow, rising limb, and falling limb of the stream hydrograph. Data from stormflow sampling were coupled with the annual river hydrographs to calculate the contribution of stormflow to the annual carbon budget. For both storm and baseflow sampling POC outflow from the reservoir was subtracted from the POC inflow to determine net POC input.

Trophic–Dynamic Analysis

The amount of carbon necessary to support the fish community in Sardis Reservoir was estimated using the equations developed by Adams et al. (1983). Fish species observed from the rotenone sampling were divided into young-of-year (YOY) and adult categories based on the body length of each species, and trophic levels were assigned to each species based on average annual diets reported by Ploskey and Jenkins (1982).

Fish standing crops for 1988 were determined by cove rotenone sampling and were corrected for recovery efficiencies reported for each species (Grinstead et al. 1977). Annual production of each species was estimated by multiplying standing crops by estimated annual mean biomass turnover rates (P/*B* ratos) (Adams et al. 1983). The organic carbon production required (CPR) to support the production of all fish species in Sardis Reservoir was then calculated as

$$\mathrm{CPR} = \sum_{1}^{n} \left(\frac{1}{E}\right)^{\tau_i} P_i$$

where CPR = total organic carbon required by the fish community, E = average trophic level conversion efficiency, τ_i = trophic level of the fish species i, and P_i = production of the fish species i.

CPR was calculated for tropic level conversion efficiencies of 10 and 15%, following the approach of Adams et al. (1983). The phytoplankton production potentially available to support the reservoir fish community (PPR) was determined using the light/dark bottle dissolved oxygen data presented previously.

Results

Meteorological Conditions

Monthly precipitation at the Sardis Reservoir dam ranged from 0.6 cm in June 1988 to 18.4 cm in September 1988 (Fig. 2). The highest monthly precipitation typically occurred from November through May. The annual changes in reservoir water level are for flood-control purposes, with the rule curve decreasing during the time immediately preceding the expected rainy season (Fig. 2). Water levels (2-wk averages) during the study period ranged from 71.9 m during the last 2 wk in December 1987 to 78.7 m during the last 2 wk of July 1987.

Fish Population Structure

The rotenone surveys indicated that gizzard shad dominated fish biomass, comprising 39.8% of the total fish biomass in the reservoir. Black basses, crappies, and catfishes (*Ictalurus* spp.) represented 13.3, 0.5, and 4.1% of the total fish biomass, respectively. Other significant species included freshwater drum (*Aplodinotus grunniens*), common carp (*Cyprinus carpio*), spotted gar (*Lepisosteus oculatus*), spotted sucker (*Minytrema melanops*), and buffaloes (*Ictiobus* spp.). The average total fish biomass was 190 $kg \cdot ha^{-1}$. The fish com-

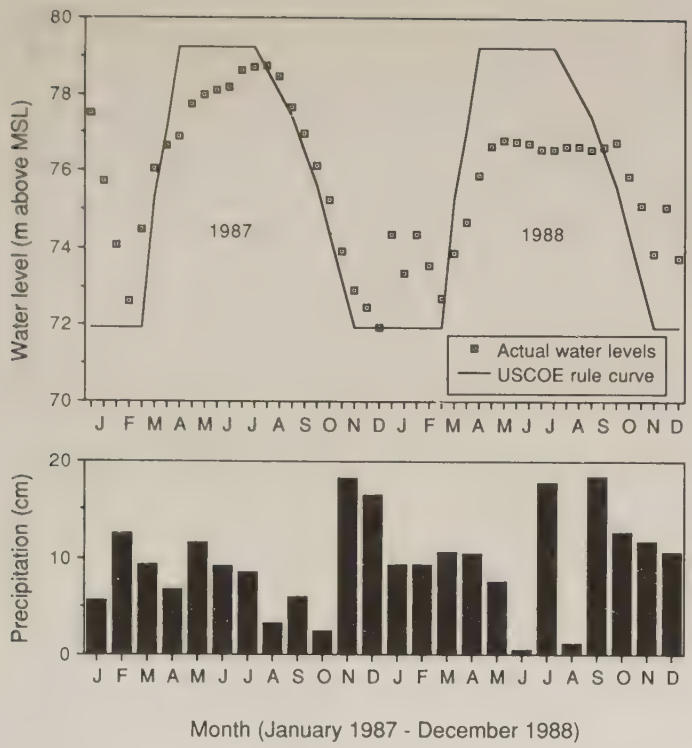
Water level (m above MSL)
80
78
76
74
72
70
1987
1988
Actual water levels
USCOE rule curve
J F M A M J J A S O N D J F M A M J J A S O N D
Precipitation (cm)
20
10
0
J F M A M J J A S O N D J F M A M J J A S O N D
Month (January 1987 - December 1988)

FIG. 2. Reservoir water level and precipitation, January 1987 through December, 1988, at Sardis Reservoir dam. Reservoir water level values are averages for 2-wk periods; precipitation values are monthly totals.

TABLE 3. Biomass of gizzard shad and largemouth bass according to centimetre size group collected by rotenone sampling in Sardis Reservoir, August 1988.

	Biomass ($kg \cdot ha^{-1}$)	
Length (cm)	Gizzard shad	Largemouth bass
5.1	3.07	0.08
7.6	1.57	0.71
10.2	0.11	1.15
12.7	0.17	0.65
15.2	1.32	0.94
17.8	2.16	0.04
20.3	1.94	0.13
22.9	3.02	1.82
25.4	28.67	3.34
27.9	27.36	8.27
30.5	6.01	3.89
33.0	0.73	0.29
35.6	0.00	0.85
38.1	0.00	0.20
40.6	0.00	0.28
43.2	0.00	0.28
48.3	0.00	0.49
58.4	0.00	1.78

munity composition reveals that largemouth bass were the primary large predator in the reservoir and gizzard shad the primary prey. Analysis of gizzard shad and largemouth bass biomass by length group, however, suggests that the majority of gizzard shad biomass is unavailable to the majority of largemouth bass biomass. This analysis is based on the assumption that largemounth bass cannot consume prey greater than one half their length (Lawrence 1958; Jenkins and Morais 1978). For example, the biomass of largemouth bass ≤30.5 cm in length is 21.02 $kg \cdot ha^{-1}$, and the biomass of gizzard shad >15 cm in length is 71.21 $kg \cdot ha^{-1}$ (Table 3).

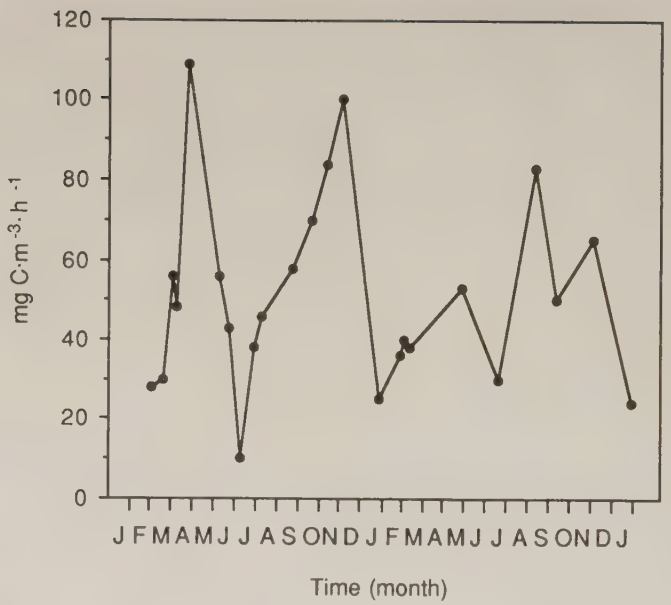

FIG. 3. Representative phytoplankton primary productivity values from Station 1, surface, from March 1987 through January 1989.

Phytoplankton Primary Production

Gross phytoplankton primary productivity averaged 31.4 mg $C \cdot m^{-3} \cdot h^{-1}$ (SD = 24.0) at all stations, depths, and sampling times over the study period. Two-way ANOVA indicated that productivity was significantly different ($F = 41.4, P = 0.0001$) among sample depths at all stations. The highest productivity rates were consistently at the surface and the lowest rates were consistently at the bottom, averaging 49.1 and 11.4 mg $C \cdot m^{-3} \cdot h^{-1}$, respectively. Secchi disk depths averaged 1.4 m. No significant differences in productivity between the two lower reservoir stations were observed, and data from both stations were pooled for subsequent analyses.

No significant differences in average productivity among seasons were observed, although seasonal trends at the surface sites were apparent (Fig. 3). Productivity exhibited autumn and spring peaks, with low values observed in July and January. No relations were observed between productivity (at all depths and locations) and Secchi disk depth, nitrate concentration, phosphate concentration, temperature, or reservoir water levels (data not shown). Seasonal trends in surface productivity did coincide, however, with consistent seasonal blooms of *Melosira* spp. that reached numbers as high as 4.4×10^5 $cells \cdot L^{-1}$ (Knight 1988). When phytoplankton productivity is calculated per square metre of vertical water column, an average of 1182 mg $C \cdot m^{-2} \cdot d^{-1}$ places Sardis Reservoir among the more productive reservoirs in the southeastern United States (Table 4).

To determine the contribution of phytoplankton primary production to the reservoir food web, productivity values were corrected for reservoir water volume. Gross primary productivity contributed an average of 2.80 Gg $POC \cdot mo^{-1}$, or an average of 33.57 Gg of POC annually, with most of the input occurring from April through October. This calculation assumed that all carbon production by phytoplankton was particulate and ignored the production of dissolved organic carbon. If POC

TABLE 4. Comparison of primary productivity values from several southeastern reservoirs (adapted from Kimmel et al. 1990).

Reservoir, location	Year	Average value (mg $C \cdot m^{-2} \cdot d^{-1}$)	Reference(s)
Oligotrophic (50–250 mg $C \cdot m^{-2} \cdot d^{-1}$)			
Canyon, Texas	1976	184	Hannan et al. 1981
DeGray, Arkansas	1979–80	199	Kennedy (referenced as unpublished data in Kimmel et al. 1990)
Mesotrophic (250–1000 mg $C \cdot m^{-2} \cdot d^{-1}$)			
Norris, Tennessee	1967	360	Taylor 1971
West Point, Alabama–Georgia	1977	689	Davies et al. 1980
Waco, Texas	1968, 1977–78	814	Kimmel and Lind 1972, Lind 1979
Texoma, Oklahoma–Texas	1979–80	934	B. L. Kimmel (referenced as unpublished data in Kimmel et al. 1990.
Eutrophic (>1000 mg $C \cdot m^{-2} \cdot d^{-1}$)			
Sardis, Mississippi	1987–88	1182	This study
Chickamauga, Tennessee	1977	1286	Placke and Poppe 1980
Moss, Texas	1976	1302	Silvey and Stanford 1978

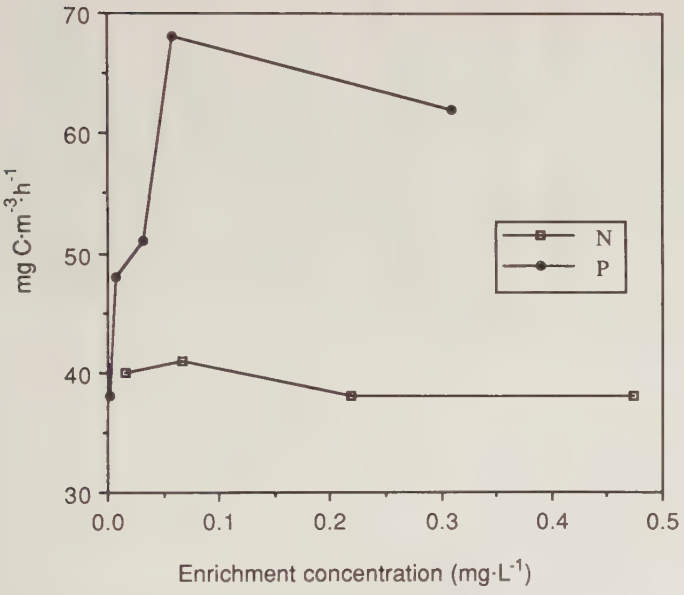

FIG. 4. Effect of nitrate and phosphate additions to surface in situ phytoplankton primary productivity incubations at Station 1, April 1987.

production by phytoplankton is corrected for the possible range of values representing percentages of carbon released as extracellular dissolved organic carbon, the production values would decrease by 17–38% (Sondergaard and Jensen 1986).

All enrichment experiments demonstrated stimulation of primary productivity by the addition of phosphate, or by the additions of phosphate and nitrate in combination. For illustration, data are presented from the April 1987 experiments only. No significant increase in primary productivity resulted from the addition of nitrate at several different concentrations (Fig. 4). Background nitrate concentrations averaged 0.051 mg $N \cdot L^{-1}$. Additions of ammonia at several different concentrations also resulted in no stimulation of productivity. Phosphate additions resulted in an increase in primary productivity over controls from 38 to a maximum of 68 mg $C \cdot m^{-3} \cdot h^{-1}$ (Fig. 4) when phosphate was increased 50 times over background (0.006 mg $P \cdot L^{-1}$). An increase in productivity was first seen with the addition of phosphate at a concentration 6.0 times over background.

Enrichments with both nitrate and phosphate alone and in combination supported the possibility that phosphate was limiting productivity. Addition of nitrate alone resulted in no stimulation of productivity, while addition of phosphate led to an increase in productivity 4.5 times that of control levels. Additions of nitrate and phosphate in combination resulted in the same level of increase that was seen with the phosphate addition alone.

With respect to vertical differences in the degree of maximum stimulation by phosphate enrichments in April and October 1987, primary productivity at surface, middle, and bottom depths increased 2.3, 3.0, and 1.7 times over control rates.

POC Input from Tributaries

The determination of POC in transport from both the Little Tallahatchie and Tippah rivers during base and stormflow indicated that VFPOM constituted the major fraction (Fig. 5). If average values for the entire study are used for the basis of calculation, VFPOM represented 79% of the total POC in transport. FPOM and CPOM represented 12 and 9% of the total, respectively.

The notable seasonal pattern of POC transport showed considerably higher amounts of VFPOM transported into Sardis Reservoir during the winter and spring months (Fig. 5). These also were months when precipitation in the drainage basin and discharge from the tributaries into the reservoir were highest and when reservoir water levels were lowest. FPOM and CPOM exhibited a similar trend, with slightly higher inputs occurring during the winter and spring months. With respect to the total input of POC to Sardis Reservoir, inflow from tributaries was responsible for an average annual input of 60.00 Gg.

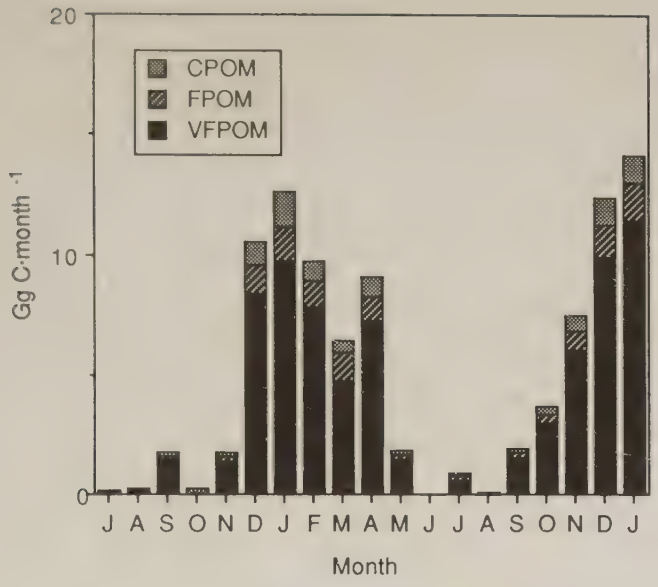

FIG. 5. POC input from tributaries, separated by size class (VFPOM, very fine particulate organic matter, 0.45–75 μm; FPOM, fine particulate organic matter, 75 μm – 1 cm; and CPOM, coarse particulate organic matter, >1 cm).

To estimate the fraction of the total POC (Fig. 6a) that was available to gizzard shad, particles too small to be filtered effectively from the water column by their gill rakers were excluded from the calculations. Microscopic measurements of VFPOM indicated that >95% of the particles were smaller than 5 μm in their greatest dimension, a size too small to be removed by gill rakers of most of the resident gizzard shad population (Mummert and Drenner 1986). Although CPOM probably is too large to be filtered effectively, it was assumed that CPOM particles eventually are reduced in size by biotic and abiotic mechanisms and become available to gizzard shad. When corrected to include only the larger particle sizes (FPOM and CPOM), the average annual input of POC dropped to 12.68 Gg. There were seasonal differences apparent in the input of this available POC, with the most transported in the winter and the least transported in the summer (Fig. 6b).

CHN analyses of POC revealed no temporal trends of C:N ratios, although there were differences apparent between POC size classes (Table 5). Two-way ANOVA indicated significant differences in C:N ratios among size fractions ($F = 29.7$, $P = 0.0001$) but not between sample collection sites. Analysis of LSD indicated that the average C:N ratio of CPOM from both collection sites (35.3) was significantly higher than C:N ratios of FPOM and VFPOM (15.0 and 10.5, respectively).

Stormflow occurring on an average of only 32 d per year during the study period was responsible for 92% of the total POC transported over the study period. An analysis of the first 36 h of one storm event that occurred in the Little Tallahatchie River drainage in September 1988 is presented as an example (Fig. 7). Approximately 11 cm of rain caused the Little Tallahatchie River to increase in stage from 2.6 to 5.3 m in just over 6 h, a dramatic response characteristic of a channelized river. Most of the POC transport occurred during the rising limb of the hydrograph, with transport rising from 0.2 to 101.1 Mg $C \cdot h^{-1}$ in 4 h (Fig. 7). VFPOM was responsible for as much as 99% of the total POC transport, increasing from a baseflow input rate of 0.15 Mg $C \cdot h^{-1}$ to a maximum of 99.8 Mg $C \cdot h^{-1}$. The input rate of FPOM responded slightly less dramatically

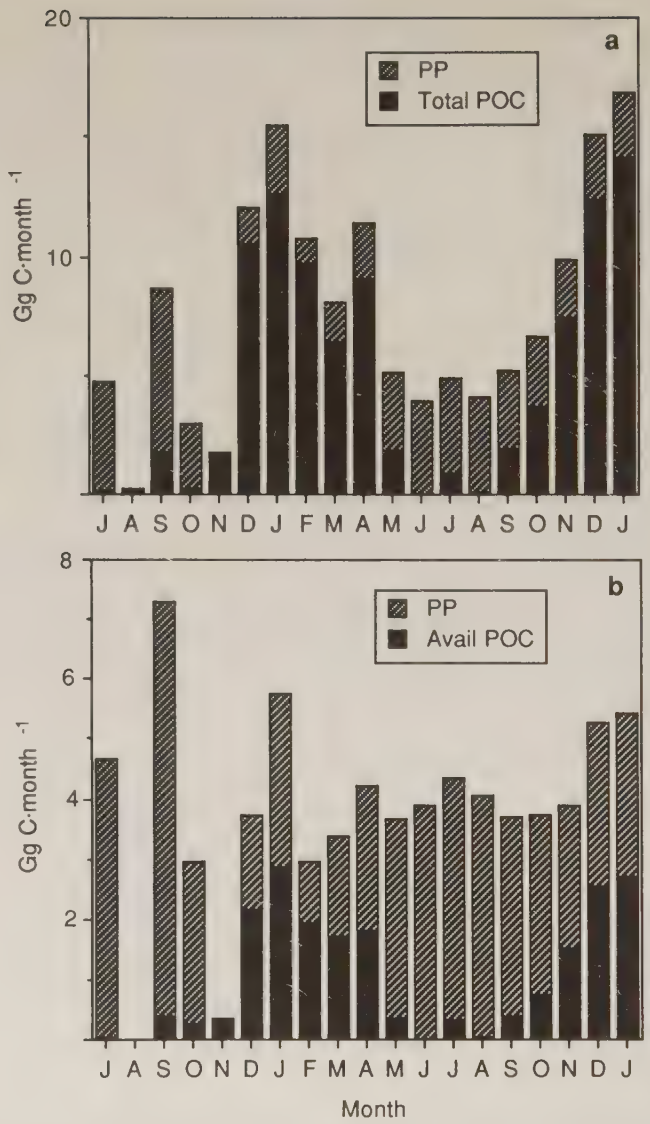

FIG. 6. (a) Total tributary POC input versus POC input from phytoplankton primary production (PP); (b) tributary POC input available to gizzard shad (>75 μm) versus POC input from phytoplankton primary production. (Phytoplankton primary production data are not available for August and November 1987.)

over the 4-h period, increasing from 0.01 to 0.27 Mg $C \cdot h^{-1}$, and CPOM increased from 0.01 to 1.01 Mg $C \cdot h^{-1}$. CPOM and FPOM transport reached their maximum levels (5.88 and 0.53 Mg $C \cdot h^{-1}$, respectively) 4 h after the VFPOM maximum.

Trophic–Dynamic Analysis

The average trophic level (τ) of all forage species was 1.78. PPR values from all sampling locations, corrected for daily photoperiod, averaged 1182 mg $C \cdot m^{-2} \cdot d^{-1}$. CPR values for 10 and 15% trophic level conversion efficiencies averaged 414 and 188 mg $C \cdot m^{-2} \cdot d^{-1}$, respectively, indicating almost a threefold greater supply of POC from phytoplankton production alone than required by the reservoir fish community.

Discussion

The fish community of Sardis Reservoir is dominated by gizzard shad, which is not unusual for southeastern reservoirs (Grinstead et al. 1977). Although fish community structure was

TABLE 5. C:N ratios of three size classes of POC collected from transport (dashes = data not available).

Date (mo/d/yr)	Little Tallahatchie River			Tippah River		
	CPOM	FPOM	VFPOM	CPOM	FPOM	VFPOM
6/11/87	29.4	8.7	51.5	42.8	7.9	7.4
6/23/87	39.1	17.5	7.9	41.2	16.3	7.9
7/7/87	24.2	18.5	6.4	22.4	9.8	8.3
7/23/87	26.8	8.3	6.2	63.7	34.5	6.9
8/11/87	23.4	10.7	16.6	49.7	24.3	7.6
9/4/87	—	19.2	7.2	—	18.7	8.2
9/22/87	37.8	11.5	8.7	40.9	12.2	7.8
10/7/87	28.7	15.7	9.3	23.6	15.3	8.4
10/20/87	—	9.3	6.3	—	11	7.1
Average	29.9	13.3	13.3	40.6	16.7	7.7

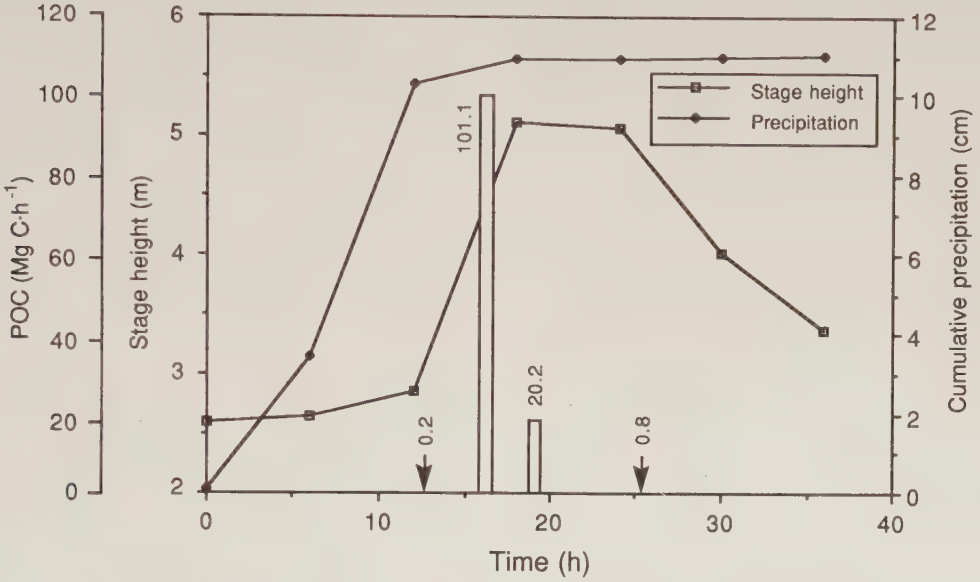

FIG. 7. Cumulative precipitation, river hydrograph, and total POC transport (indicated by vertical bars and numbers) for the first 36 h of a September 1988 storm event at the Little Tallahatchie River inflow station.

assessed on only one sampling date in this study, results agree closely with results from sampling in previous and subsequent years (Meals and Kiihnl 1987, 1990, 1991). Because of their abundance and availability as prey for predators such as largemouth bass, gizzard shad may serve as the primary link between lower and higher tropic levels in the reservoir. Adult gizzard shad can provide this link by filter feeding on plankton or plant-derived POC in suspension or on POC from the surface layers of sediments (Baker and Schmitz 1971; Mummert and Drenner 1986; Mundahl 1988). Mundahl and Wissing (1988) recently suggested that gizzard shad may exhibit some degree of selectivity when feeding on detrital POC in surface sediments.

Zooplankton rarely have been observed as part of age 0 and adult gizzard shad diets in Sardis Reservoir, however, which is similar to the findings of Mundahl and Wissing (1987) in an Ohio reservoir. In gut content analyses of age 0 and adult gizzard shad collected semiannually from Sardis Reservoir from 1987 to 1989, zooplankton were found only once (April 1989), and then comprised only 0.3% frequency by number of the total organic diet (Crist 1988; Miller 1990). Zooplankton tows conducted at various depths at Stations 1 and 2 in Sardis Reservoir in April, July, and September 1988 revealed low numbers of zooplankton (1–167 organisms·L^{-1}) with as much as 72% of the total numbers consisting of small protozoans (D. E. Miller, unpubl. data). Further study is required to determine if zooplankton are being suppressed in Sardis Reservoir by the large gizzard shad population, particularly the larvae (Kutkuhn 1955; Drenner et al. 1986).

The absence of zooplankton in gizzard shad diets may increase the relative importance of detritus to their nutrition. The amount of detritus in gizzard shad diets may be high, comprising as much as 99% of their gut contents (Baker and Schmitz 1971). Coincidentally, gizzard shad usually are found in much higher numbers in the upper reaches of reservoirs (Netsch et al. 1971; Storck et al. 1978), where the concentrations of particulate detritus are the highest. If gizzard shad indeed obtain nutritional benefit from detritus, then the supply of detritus from mudflat primary production might be significant to them. Although reservoir drawdown usually is considered detrimental to gizzard shad populations because of decreased spawning habitat and cover from predators, benefits may exist. Vascular plants growing on exposed mudflats during drawdown later could provide a source of detrital POC as increasing reservoir water levels inundate the vegetation and decomposition occurs (Aumen et al. 1991).

In contrast with their role as prey for other fish, gizzard shad may compete with other fish for food resources. Using data

from 1988 as a specific example, 83% of the largemouth bass biomass was in fish ≤30.5 cm in length. Assuming that largemouth bass cannot consume prey greater than one half their length (Lawrence 1958; Jenkins and Morais 1978), the effective prey length for this bass population was ≤15 cm. Using these constraints on predation, 94% of the forage fish community biomass in 1988 was larger than a size available as prey to 83% of the largemouth bass community biomass (Table 3). Because of this abundance of gizzard shad too large to be consumed by many other predators, examination of gizzard shad trophic requirements and factors controlling their population size becomes important to the consideration of the overall reservoir ecology.

A potential source of POC to the reservoir food web is phytoplankton primary productivity. Sardis Reservoir can be classified as slightly eutrophic, with an average productivity of 1182 mg $C \cdot m^{-2} \cdot d^{-1}$ (Table 4). Warmwater reservoirs located within highly erodible agricultural watersheds have the potential to be very productive (Kimmel et al. 1990); thus, it is not surprising that Sardis Reservoir is productive. The average Secchi disk depth of 1.4 m indicates that light penetration is sufficient in this shallow reservoir to allow photosynthesis to occur throughout a large portion of the water column. The paucity of phosphate in the reservoir and the indication that it can limit primary production suggest low levels of phosphate loading, even though the degree of turbidity indicates that erosion is occurring within the watershed. However, present-day erosion probably is occurring on relatively nutrient-poor soils (U.S. Department of Agriculture 1988). The highest rates of erosion observed in the country occurred in this region during the early part of this century, and it is likely that most of the nutrient-containing topsoil was lost during that time.

The calculation of phytoplankton productivity per square metre of vertical water column has some limitations when comparing trophic status between reservoirs of varying depth and when predicting fish production. For example, a deep, eutrophic reservoir with an extensive aphotic zone would have very little primary production over much of its depth. Another shallow reservoir of much lower trophic status with the euphotic zone extending to the bottom might exhibit the same primary productivity estimate per square metre of water column.

Data for this study were collected when reservoir water levels were 2–4 m below normal because of drought. Subsequent to the phytoplankton primary production data collection, high rainfall increased water levels in Sardis Reservoir by almost 10 m in the summer of 1989. It is doubtful that PPR estimates calculated as milligrams carbon per square metre per day would have been truly comparable between these low- and high-water periods. Comparisons of trophic status and fish production using only the euphotic zone would be more realistic but were not possible during this study because of the unavailability of a submersible light sensor.

The high levels of spring and autumn productivity that occur in the surface samples may be considered usual for warmwater lentic systems (Wetzel 1983). Temporal variability in productivity, however, cannot be related to physical/chemical factors that were measured as part of this study. In particular, it is surprising that although the nutrient enrichment experiments indicated phosphate limitation of primary productivity, year-round variations in productivity were not related to phosphate concentrations in the water column. It is likely that productivity is regulated temporally by a combination of factors such as nutrient availability, temperature, light availability, and phytoplankton species composition.

The concidence between *Melosira* blooms and peaks in productivity at the surface sample sites suggests that this organism may be responsible for the majority of the algal productivity. Silica was not measured as part of this study and may be a factor that has considerable influence over diatom populations and total phytoplankton productivity in the reservoir.

Results of the enrichment experiments indicate that increases in phosphate concentration are capable of stimulating phytoplankton productivity. The lack of stimulation by nitrate and ammonia enrichments is not surprising, given the abundance of nitrogen in the reservoir, and ruled out the possibility of nitrogen limitation, at least in the spring and autumn. Photoinhibition at the surface may have limited productivity even in the presence of additional phosphate, and the bottom depths may have been limited by the lack of light rather than phosphate availability.

Another large source of organic matter for the reservoir food web is POC transported into the reservoir from inflowing tributaries. Most of the FPOM and CPOM input from inflowing tributaries appears to be detritus formed by decomposition of terrestrial vegetation. Visual analysis of the larger particle sizes revealed that morphous plant material with recognizable plant cells, such as tracheids, is frequently apparent. The dominant size fraction of detrital input, however, is VFPOM. This observation is not surprising, given the propensity of VFPOM to remain in suspension, even during the lowest stream velocities observed during baseflow conditions. Larger POM requires more energy to be moved from sediments or bedload into transport.

The VFPOM fraction consists largely of amorphous particles that are unidentifiable as to origin. Because of the very small size of this POM fraction, it is possible that this material has already been acted upon substantially by physical and biological processes, leaving behind only those chemical components most resistant to decay. This size fraction might represent a carbon substrate that is poor in quality relative to unprocessed organic matter, although its low C:N ratios relative to CPOM would seem to indicate higher quality. Rice (1982) has suggested, however, that low C:N ratios may not necessarily represent high quality. The nitrogen represented in the C:N ratio may be nonproteinaceous and indigestible by many detritivores (Odum et al. 1979; Bowen 1980; Rice 1982). Relative amounts of nitrogen associated with humic substances generally increase during the aging process (Rice 1982); thus, the VFPOM and FPOM may have been aged longer than CPOM and may be of poor food quality.

An alternative hypothesis concerning the formation of VFPOM is that the particles are formed primarily by precipitation of dissolved organic matter and may be of higher nutritional quality than VFPOM formed by the process described previously (Bowen 1984). Various physical, chemical, and biological factors convert dissolved organic matter to particles (Dahm 1981; Bowen 1987), with sizes ranging from 0.5 to 250 μm and having no cellular structure (Mann 1988). These particles have been found to comprise over 60% of sediment organic matter and may be a relatively nutritious food source (Bowen 1984).

The largest inputs of POC from tributaries occur during the late winter and spring months when rainfall is highest and the reservoir is at its lowest annual elevation. The average retention time of water in the reservoir also is at its lowest point at this time, making the reservoir more riverine than lacustrine (Kimmel et al. 1990).

Also in keeping with the riverine nature of the reservoir under such conditions, the majority of POC transport, particularly VFPOM, occurs during stormflow. The principal tributaries flowing into the reservoir have been channelized and respond rapidly to storm runoff. Most of the POC input during storms occurs during the rising limb of the hydrograph (Fig. 7). The differences in timing of VFPOM, FPOM, and CPOM inputs relative to the storm hydrograph also are informative. VFPOM increases almost instantaneously with increasing river stage, indicating that this material is transported readily. CPOM and FPOM transport reached their maximum 4 h later than VFPOM, indicating that the principal source of this material is the river bank and floodplain. CPOM and FPOM is transported when the increasing river level inundates and removes terrestrial detritus.

If all of the potential sources of POC to the reservoir food web are considered together, i.e. phytoplankton primary production, input from tributaries, and mudflat primary production, POC input from tributaries represents 55.0% of the total. The potential annual contribution of mudflat primary production is 15.5 Gg of C (Aumen et al. 1991). Phytoplankton primary production and mudflat primary production constitute 30.8 and 14.2% of the total, respectively.

The dominance of allochthonous sources of POC to Sardis Reservoir over autochthonous sources is expected for a relatively well-flushed reservoir (Groeger and Kimmel 1984; Kimmel et al. 1990). However, if only that portion of the POC input that is available directly to the reservoir food web via gizzard shad consumption is considered, phytoplankton primary production dominates as the primary source of organic matter to the food web. The importance of POC input from tributaries decreases to only 20.5% of the total, while the importance of phytoplankton and mudflat primary production increases to 54.4 and 25.1%, respectively. Although phytoplankton production predominated on an annual basis, seasonal shifts in POC input could have a major influence on the quality of POC available to the reservoir food web, particularly the detritivorous component.

If the gizzard shad diet contained zooplankton, the importance of POC input from tributaries to gizzard shad would increase. Zooplankton are capable of feeding on very small particles (Wetzel 1983), and thus, VFPOM would contribute indirectly to resources available to gizzard shad.

The timing of the different POC inputs also is relevant to the gizzard shad population, particularly during spawning season. Gizzard shad in Sardis Reservoir spawn mostly in April (Miranda and Muncy 1987) and might benefit from a high-quality carbon source in the months just preceding this spawning period. However, the potential for this benefit might be offset by low water temperatures and suppressed feeding activity. A comparison of the annual pattern of POC input from phytoplankton production and tributary input reveals that the available POC in transport is relatively high with respect to phytoplankton production just before spawning (Fig. 6). It is likely, however, that because of the absence of lignin and cellulose, phytoplankton primary production represents a food source of higher quality and nutritional value than vascular plant primary production from terrestrial sources. Chemical analyses of detritus in transport and feeding studies of gizzard shad are needed to determine the influence of these possible differences.

Based on the trophic–dynamic analysis, there is more than sufficient phytoplankton production to support the reservoir fish community, regardless of whether the calculations are based on 10 or 15% trophic-level conversion efficiencies. However, artificially high PPR values relative to food web analysis could result if a substantial portion of Sardis Reservoir's phytoplankton is composed of nannoplankton too small to be utilized directly by most filter-feeding fish. Alternatively, artificially high PPR values could result if a substantial fraction of primary production is going into dissolved organic carbon production not utilizable directly by filter feeders.

High PPR values also could result from the use of gross primary productivity measurements rather than net primary productivity measurements. Although not measured routinely in Sardis Reservoir, net primary productivity was slightly lower than gross primary productivity and would not have changed the overall results substantially if it had been used. For example, in May 1987, gross and net primary productivity at the surface of Station 1 was 56 and 55 mg $C \cdot m^{-3} \cdot h^{-1}$, a difference of only 2%. Differences were even less at middle and bottom depths (C. L. Crist, unpubl. data).

When compared with data presented by Adams et al. (1983) for 17 southeastern and midwestern reservoirs, Sardis Reservoir has the smallest CPR:PPR ratio (0.35 and 0.16 for 10 and 15% conversion efficiencies, respectively). A ratio of 1.0 indiates that PPR can account for all of the organic carbon required to support the estimated fish production. Ratios greater than 1.0 suggest that PPR does not provide sufficient organic carbon to sustain fish production, and conversely, ratios less than 1.0 suggest that PPR exceeds the organic carbon required. Assuming a 10% trophic level conversion efficiency, Adams et al. (1983) showed that most of the reservoirs they examined have insufficient levels of organic carbon produced by phytoplankton. However, with a 15% conversion efficiency, PPR was sufficient to support the fish community production in 10 of the 17 reservoirs examined.

Why Sardis Reservoir is so different from these other reservoirs is not clear from this study alone. Assuming that the PPR estimate is valid, the data indicate that either the carbon transfer mechanisms from primary producers to higher trophic levels are inefficient or that fish community production is limited by factors other than carbon, such as availability of habitat or spawning sites, competition, predation, or overfishing.

An obvious consideration with respect to limitation of fish biomass is the high fishing pressure to which Sardis Reservoir is subjected. Again using 1988 data, largemouth bass, white crappie, and black crappie represented 22.2% of the reservoir fish community CPR. Even if reduced fishing pressure increased bass and crappie CPR values by 100%, that only would increase the total CPR by 18%, a value too small to dramatically change the PPR:CPR ratio with respect to the reservoirs considered by Adams et al. (1983).

The average trophic level (τ) of these forage species is 1.78 (Ploskey and Jenkins 1982), indicating that they are feeding mostly on materials associated with plants (phytoplankton or detritus). Although many of these fish utilize material at the base of the food web, they divert 36% of the organic carbon required for the entire fish community into their biomass, which is considered undesirable from a management standpoint. Due to the diversion of carbon into this fraction of the fish community, the carbon flow to higher tropic levels is uncoupled (Fagan and Fitzpatrick 1978). Incomplete carbon transfer to higher tropic levels may be responsible for the low CPR:PPR ratio of Sardis Reservoir.

Several paradoxes exist with respect to the major sources of POC to the food web of Sardis Reservoir. The trophic–dynamic analysis suggests that it is not necessary to invoke allochthonous sources of POC to support the observed fish community.

However, our research and others (Baker and Schmitz 1971; Miller 1990) show that the dominant forage fish (gizzard shad) consistently have large amounts of allochthonous detritus in their guts. In a laboratory feeding study examining the potential for gizzard shad to assimilate detrital carbon collected from Sardis Reservoir, Miller (1990) demonstrated that gizzard shad apparently can assimilate protein from detritus, agreeing with a similar observation by Mundahl and Wissing (1988). It is not clear why detritus, commonly considered to be of poor nutritional quality, should comprise a significant portion of gizzard shad diet in Sardis Reservoir where there is abundant phytoplankton production.

The results of this study support the assertion of Adams et al. (1983) that research and management strategies directed at enhancement of the production or standing crop of smaller forage fish may be the best approach to developing larger sportfish (predator) populations. A more efficient transfer of carbon through the food web might be accomplished by stocking Sardis Reservoir with a smaller forage fish, such as threadfin shad, (*Dorosoma petenense*), which are currently not found in the reservoir. Threadfin shad are generally smaller than gizzard shad and are of a size more suitable for predation by bass. Threadfin shad have been introduced occasionally into Sardis Reservoir, although none has survived the winter (C. A. Schultz, MDWFP, P.O.Box 451, Jackson, MS, pers. comm.). Even though introduced threadfin shad may require more zooplankton in their diet than gizzard shad and might compete with other fish for resources, the low CPR:PPR ratio indicates that sufficient resources may be available to support annual introductions of this forage species. This management strategy could increase the amount of carbon fixed by reservoir primary production that ultimately reaches the higher trophic levels.

In addition, preliminary results from harvest restrictions on black basses and crappies indicate restructuring of the gizzard shad population to include more small and intermediate size groups and the largemouth bass population to include more large size groups (Meals and Kiihnl 1991). It is anticipated that the biomass of predators will increase as more food becomes available to them.

Acknowledgements

This study was supported by Dingell-Johnson Federal Aid Project F-78 through the Mississippi Department of Wildlife, Fisheries, and Parks. Matching funds were provided by the Office of Research, University of Mississippi. The authors gratefully acknowledge assistance in project design and execution provided by all MDWFP personnel. We are also indebted to Peng Lee, Paul Katnik, Robin Bennett, Traci Bennett, Margaret Ko, and Christy Ko for field and laboratory help, to Stacy Jo Linder for preparation of figures, and to Gary Miller and three anonymous reviewers for helpful comments on the manuscript. We thank the U.S. Army Corps of Engineers, Vicksburg District, Vicksburg, MS, for access to the Environmental Data Transmitter Network and the Agricultural Weather Service Center, Stoneville, MS (NWS, NOAA, DOC), for solar radiation data.

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PERSPECTIVES

Historic Changes in Lake Michigan Zooplankton Community Structure: The 1960s Revisited with Implications for Top-Down Control

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Evans, M. S. 1992. Historic changes in Lake Michigan community structure: the 1960s revisited with implications for top-down control. Can. J. Fish. Aquat. Sci. 49: 1734–1749.

Recent changes in the Lake Michigan ecosystem provide a benchmark against which to reevaluate historic data. During the 1960s, the alewife (*Alosa pseudoharengus*) population exploded and then crashed. Offshore zooplankton data for the summers of 1954, 1966, and 1968 provided evidence that variations in alewife abundance had a major effect on zooplankton community structure. Based on these observations, other researchers have hypothesized that increased and decreased phytoplankton abundances during the 1960s as recorded at the Chicago water filtration plant were due to top-down effects rather than to phosphorus loading. This argument is reevaluated using two approaches. First, from the relationship between interannual variability in alewife and zooplankton species abundance during the summers of 1954, 1966, 1968, 1977, 1982, and 1984–87, I conclude that the effects of alewife predation on zooplankton community structure during the 1960s are less clear then originally proposed. Second, from estimates of *Daphnia* spp. grazing rates, considerations of the source of the long-term phytoplankton data used to support the top-down argument, regional differences in phytoplankton, zooplankton and alewife abundance trends, and historic water clarity observations, I conclude that existing data are insufficient to support the top-down argument that long-term trends in phytoplankton abundance were primarily affected by fluctuations in alewife abundance.

Les changements récemment apparus dans l'écosystème du lac Michigan constituent un jalon à partir duquel on peut réévaluer les données antérieures. Pendant les années 60, la population de gaspareau (*Alosa pseudoharengus*) a explosé pour ensuite s'effondrer. Les données sur le zooplancton pélagique pour les étés 1954, 1966 et 1968 ont montré que les variations dans l'abondance du gaspareau avaient un effet très important sur la structure de la communauté zooplanctonique. À partir de ces observations, d'autres chercheurs ont posé que la hausse et la baisse de l'abondance du phytoplancton dans les années 60, qui ont été enregistrées à l'usine de filtration de l'eau de Chicago, étaient dues à des effets trophiques descendants plutôt qu'à une charge en phosphore. Cet argument est réexaminé selon deux démarches. Tout d'abord, à partir de la relation entre la variabilité annuelle de l'abondance du gaspareau et des espèces zooplanctoniques pendant les étés 1954, 1966, 1968, 1977, 1982 et 1984–1987, je conclus que les effets de la prédation du gaspareau sur la structure de la communauté zooplanctonique dans les années 60 sont moins clairs qu'on ne le croyait au départ. Deuxièmement, à partir des estimations du taux de broutage de *Daphnia* spp., de l'examen des sources des données à long terme sur le phytoplancton qui servaient à étayer l'argument sur l'effet trophique descendant, des différences régionales dans les tendances de l'abondance du phytoplancton, du zooplancton et du gaspareau et enfin d'observations recueillies depuis longtemps sur la transparence de l'eau, je conclus que les données existantes sont insuffisantes pour étayer l'argument de l'effet trophique descendant selon lequel les tendances à long terme de l'abondance du phytoplancton étaient affectées avant tout par les fluctuations de l'abondance du gaspareau.

Received June 20, 1991
Accepted April 6, 1992
(JB092)

Reçu le 20 juin 1991
Accepté le 6 avril 1992

Lake Michigan is distinctive, not only by virtue of its large size (sixth among the world's lakes), but because many of the changing limnological properties of the lake have been relatively well documented. The most detailed records are for fish and phytoplankton populations. In contrast, long-term records for zooplankton and benthos abundances and concentrations of plant nutrients such as nitrite-nitrate and orthophosphorus are more limited. Thus, although many differences have been noted in the limnological features of Lake Michigan over the years, the causal factors affecting such changes are often poorly understood and hence subject to some debate.

Annual records for commercial fish catches extend back to the 1880's (Wells and McLain 1973). Annual records for forage fish abundance extend back to 1963 when the U.S. Fish and Wildlife Service began its annual autumn fish-census program (Eck and Wells 1987). Long-term trends in fish abundances have been related to overfishing, habitat alterations, the sea lamprey (*Petromyzon marinus*) invasion, the invasion of exot-

ics such as the alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*), salmonid stocking programs, and climatic variability (Wells and McLain 1973; Christie 1974; Eck and Brown 1985; Eck and Wells 1987; Coble et al. 1990).

Long-term sampling at municipal water intakes has documented the increased concentration of total dissolved solids, sulfate, and chloride in Lake Michigan inshore waters over 1870–1970 (Beeton 1969). These trends have been related to increased population and technological growth in the Great Lakes drainage basin (Beeton 1969). Other long-term changes in the chemical composition of Lake Michigan waters have been the subject of some debate. For example, the apparent decrease in soluble reactive silica concentrations over the first half of this century has been related to increased phosphorus loading (Schelske and Stoermer 1971; Schelske et al. 1986; Schelske 1988) and to changes in analytical methodology (Torrey 1976; Shapiro and Swain 1983).

Long-term trends in phytoplankton abundances have been best documented through the routine water quality analyses conducted at the Chicago and Milwaukee water filtration plants. While there has been some discussion of the factors affecting long-term trends in phytoplankton abundance at the Milwaukee water filtration plant (Brooks et al. 1984; Edgington 1984, 1986), more comprehensive analyses have been conducted for phytoplankton abundance trends at the Chicago water filtration plant (Damann 1960; Danforth and Ginsburg 1980; Makarewicz and Baybutt 1981; Brooks et al. 1984; Edgington 1984, 1986). These studies have determined that phytoplankton abundances were low over the late 1920s to early 1950s and increased in the mid-1950s and 1960s. Abundances remained high from the late 1960s through the early 1970s and then declined precipitously through the mid-1970s to values observed during the 1920s. Increased algal abundances through the 1950s and 1960s have been attributed to accelerated eutrophication through increased phosphorous loadings (Schelske and Stoermer 1971; Chapra and Robertson 1977): decreased abundances through the early to mid-1970s have been attributed to the implementation of phosphorus abatement programs (Danforth and Ginsburg 1980; Makarewicz and Baybutt 1981). Others hypothesize that long-term variations in phytoplankton abundances at the Chicago water filtration plant can be attributed to variations in the abundance of planktivorous fish which in turn influenced the intensity of zooplankton grazing pressure (Brooks et al. 1984; Edgington 1984, 1986).

Several studies conducted in small ponds and mesocosms have shown that top-down control mechanisms can be effective in controlling algal abundance, i.e. reductions in the abundance of planktivorous fish allow large-bodied zooplankton to predominate which then exert intense grazing pressure on the phytoplankton community. This results in a concomitant decrease in algal standing stocks and an increase in water clarity (Lynch and Shapiro 1981; Shapiro and Wright 1984; Carpenter et al. 1985). The potential effectiveness of top-down control mechanisms for improving water clarity in larger systems is unknown but recently has gained much attention for the Lake Michigan ecosystem (Scavia et al. 1986: Kitchell et al. 1987).

Recent support for the hypothesis that top-down mechanisms may be effective in controlling algal abundance in Lake Michigan was based on a series of studies conducted during the 1970s and early 1980s. These studies documented a decline in alewife abundances through the 1970s and a concomitant increase in the relative dominance of the large-bodied *Daphnia galeata mendotae* over the smaller-bodied *Daphnia retrocurva* (Jude and Tesar 1985; Evans and Jude 1986). As alewife abundances continued to decline into the early 1980s, the large-bodied *Daphnia pulicaria* became established in the offshore zooplankton community (Evans and Jude 1986). By summer 1983, it dominated the offshore zooplankton biomass. Moreover, chlorophyll concentrations were lower and water clarity was markedly higher in summer 1983 than in earlier summers (Scavia et al. 1986). *Daphnia pulicaria* continued to be a major component of the summer zooplankton biomass in 1984; water clarity remained high and chlorophyll concentrations low. This improved water clarity appeared to be a consequence of top-down controls (Scavia et al. 1986). However, later studies of zooplankton–phytoplankton relationships in this region of Lake Michigan have been less supportive of the top-down hypothesis (Dorazio et al. 1987; Lehman 1988).

Top-down control also has been implicated as the causal factor affecting increased phytoplankton abundances in Lake Michigan during the 1960s. Briefly stated, it has been hypothesized that increased alewife abundances in the late 1950s and early 1960s resulted in a decrease in the abundance of large-bodied zooplankton grazers; phytoplankton increased in abundance with this decrease in grazing pressure. Alewife abundances crashed in 1967 and remained low in 1968 with some recovery in later years. It has been hypothesized that reduced alewife abundances following the 1965–66 population explosion allowed large-bodied zooplankton to increase in abundance, grazing pressure on the phytoplankton community to intensify, and algal standing stocks to decrease (Brooks et al. 1984; Edington 1984, 1986).

One of the major limitations to the evalution of the top-down hypothesis is the lack of detailed long-term records of zooplankton abundances for the period prior to the 1970s. Zooplankton data used to support this hypothesis are based entirely on a study conducted by Wells (1970) in which he compared offshore zooplankton abundances during three summers: in 1954 when alewife were extremely rare, in 1966 at the height of the alewife population explosion, and 1968 following the alewife population crash.

Interannual variability in total zooplankton abundances for a given month can be large, with the coefficient of variation exceeding 95% for summer months (Evans and Sell 1983). Furthermore, there may be more year-to-year variation in zooplankton abundances within two relatively short study periods than between longer study periods. For example, Pedros-Alio and Brock (1985) determined that interannual variability in zooplankton abundance was greater within two periods, 1894–96 and 1976–80, than between the two study periods. Interannual variability in zooplankton abundance may be affected by a number of environmental factors in addition to size-selective fish predation, e.g. algal abundance, algal composition, invertebrate predation, temperature, etc. Thus, it is not clear how much of the variation in taxa abundances observed by Wells (1970) was associated with inherent variability beween years and how much variation was associated with major changes in zooplankton abundances. Nor is it clear how much of this variation was due to fluctuations in alewife abundance.

The tacit assumption both with Wells' original study and later arguments based on his study (Edgington 1984, 1986) is that differences in zooplankton abundances between years represented major changes in zooplankton community structure. Moreover, it was assumed that the major factor affecting these differences in zooplankton abundances was variability in alewife abundances. By making this assumption, researchers have been able to infer long-term changes in the intensity of grazing pressure on the phytoplankton community based on records of

long-term changes in alewife abundance (Brooks et al. 1984; Edgington 1984, 1986).

The purpose of this paper is to reexamine the argument that increased algal abundances in the Chicago region of Lake Michigan during the 1960s were directly associated with increased alewife abundances. This evaluation will be accomplished in two stages.

First, I compare interannual variation in zooplankton abundances as observed by Wells (1970) with the interannual variation in zooplankton abundance between the summers of 1977, 1982, and 1984–87. Interannual variability in zooplankon abundance across the entire study period (i.e. 1954–87) is then compared with interannual variations in alewife abundance; this allows for an assessment of the possible role of variation in alewife predation in affecting interannual differences in zooplankton species abundance as originally discussed by Wells (1970) and for later periods.

Second, I examine other assumptions of the top-down argument and identify other weaknesses in this hypothesis. Various insufficiencies in the hypothesis include limitations in the phytoplankton and alewife data used in the top-down argument, considerations of inshore–offshore and other regional differences in ecosystem structure, estimates of *Daphnia* grazing pressure, and considerations of other environmental variables which may have affected interannual differences in zooplankton abundance as reported by Wells (1970).

I also present historic Secchi disc data for the period when alewife were most abundant, i.e. the early to mid-1960s when water clarity presumably was the lowest. As these water clarity observations were made in the offshore region, they allow for the evaluation of the relationship between water clarity, increasing alewife abundances, and possible changes in zooplankton grazing pressure as discussed by Edgington (1984, 1986). Moreover, these Secchi disc data allow for the further evaluation of the significance of the apparently improved water clarity in the offshore region during the summers of 1983 and 1984 as discussed by Scavia et al. (1986).

Materials and Methods

Field and Laboratory Methods

Methods used for sampling zooplankton in 1954, 1966, and 1968 are described in Wells (1970). In brief, zooplankton were collected at a 74-m-deep station located 12.8 km offshore of Grand Haven, Michigan. Clarke-Bumpus plankton samplers equipped with 366-μm-mesh nets were used to sample zooplankton at the surface and at 10, 20, 30, and 40 m (or a subset of these depths). Samples were collected in mid-July and early August in the three study years. Tows were made over four time intervals, ranging from early evening to an hour before or after midnight (Wells 1970).

In July and August 1977, zooplankton samples were collected at a 100-m-deep station located approximately 20 km offshore of Grand Haven. Data used in this paper were collected at 10-m depth intervals between 0 and 40 m by using a vertically hauled closing net equipped with a calibrated flowmeter. In July and August 1982, 1984, 1986, and 1987, zooplankton were collected at this 100-m station by hauling a plankton net from 40 m to the surface: replicate samples were collected. In July and August 1985, collections were made at an 80-m station offshore of Grand Haven. A 50-cm-diameter, 156-μm-mesh net was used. Since adult copepods considered in this paper are large bodied, they were as effectively captured by the 76-μm-mesh net as by the coarser 156-μm-mesh nets used during most of the study (Evans and Sell 1985). The use of a flowmeter allowed for corrections of differential filtering efficiencies. All collections were made during daylight hours.

Counting and identification procedures for zooplankton collections made in 1954, 1966, and 1968 are described in Wells (1960, 1970). In brief, adult cladocerans, cyclopoids, and calanoids were identified to species and, where feasible, mean lengths determined. With the exception of the diaptomids, estimates of adult copepod abundances were based on the identification and enumeration of both males and females. Abundances of adult *Diaptomus* spp. were based on the identification and enumeration of males only (Wells 1960).

Counting and identification procedures for zooplankton samples collected in 1977, 1982, and 1984–87 are described in detail in Evans and Jude (1986). In brief, adult copepods and cladocerans were identified to species. Both male and female *Diaptomus* spp. were identified and enumerated.

Data Presentation and Comparisons

In order to compare zooplankton data collected by Wells (1970) in 1954, 1966, and 1968 with my later collections (1977, 1982, and 1984–87), I calculated mean summer (mid-July and early August) taxa abundances based on data presented in his tables 3 and 4. In previous work, I have used similar calculations of mean summer abundances to follow long-term trends in zooplankton abundance, biomass, and water clarity (Evans 1986, 1990; Evans and Jude 1986; Scavia et al. 1986). Adult *Diaptomus* abundance estimates for 1954, 1966, and 1968 were multiplied by 2 since Wells' abundance estimates were based on males only. Male to female ratios for adult diaptomids tend to approximate 1:1 during summer (M. S. Evans, unpubl. data).

Wells used coarser nets (366-μm mesh) than in my study (156- and 76-μm mesh), possibly resulting in underrepresentation of small- and medium-bodied zooplankton including *Diaptomus* spp. and *Cyclops* spp. adults and *Bosmina longirostris* (Wells 1970; Evans and Sell 1985). However, because the Clarke-Bumpus nets were towed for 10 min over a substantial distance (1.1 km), clogging may have occurred, reducing some of this potential bias. This reduction in bias would not have been constant for all samples but would have varied with depth, month, and year as a function of vertical gradients, seasonality, and interannual differences in phytoplankton abundance. Wells (1960) first attempted to use 156-μm-mesh nets in his 1954 investigations, but because of severe net clogging, he redesigned his sampling protocol to use coarser 366-μm-mesh nets.

Wells' (1970) data are, for the most part, based on collections made from early evening to around midnight. In contrast, my collections were made during daylight hours. Thus, my studies may have underestimated the abundances of zooplankton species which are concentrated below 40 m during daytime and migrate into this upper depth strata during nighttime. This vertical migration is likely to be most important for *Limnocalanus macrurus* and *Senecella calanoides*. Both species are hypolimnetic zooplankters, exhibiting a strong nighttime migration up into shallower regions of the water column (Wells 1960; M. S. Evans, unpubl. data).

Long-Term Trends in Alewife Abundance

Long-term trends in alewife abundance are based on data collected by the U.S. Fish and Wildlife Service during their annual autumn lakewide census of forage fish abundances and presented during the Annual Great Lakes Fishery Commission

Meeting. Alewife data presented by Wells (1985) were reported as changes in relative abundances for 1960–84: data presented by Brown (1990) were in the form of changes in catch-per-unit-of-effort for 1973–89. Based on these two studies, I present long-term trends in relative abundance for the 1954–87 period. The year of maximum alewife abundances, 1966, is assigned a value of 1.0. Abundance estimates are based on the catch of age 1 and older alewife. The year 1954 was assigned a value of 0.001 based on the fact that while alewife were widespread in Lake Michigan by 1953, they were not commonly observed lakewide until1956 (Wells and McLain 1973).

Statistical Analyses

The nonparametric Pearson's product moment correlation (Conover 1980) was calculated to investigate the relationship between alewife relative abundance and zooplankton species abundance for the nine study summers. A nonparametric test was used because of the small number of observations comprising this data set. Moreover, 1966 was a year of exceptionally high alewife abundances and markedly different from the other years considered: a parametric regression analysis would have been inappropriate because the 1966 observations would have had a disproportionately large effect on the outcome of the analysis. Nonparametric analysis, by converting abundance estimates to ranks, circumvented this problem. Species which occurred only sporadically in the plankton were not included in the correlation test because the large number of zero values precluded meaningful statistical analyses.

Although the majority of the results section focuses on the analysis of Wells' (1970) and my later data collected in the region offshore of Grand Haven, I also performed a second series of analyses based on zooplankton data collected in the offshore region of southeastern Lake Michigan over the summers of 1972–81. These analyses were performed for four reasons. First, with the exception of 1966, the years considered in my initial analysis of the relationship between long-term trends in zooplankton and alewife abundances were years of relatively low alewife abundances (relative abundance = 0.001–0.29). By including this second zooplankton data set in my analyses, I was able to examine the relationship between zooplankton and alewife abundances during the early 1970s when alewife abundances were higher. Second, by conducting these analyses based on data collected in another region of the lake, I was able to perform a second test of the hypothesis that long-term changes in alewife abundance have had significant effects on long-term changes in zooplankton species abundance in Lake Michigan. Third, because alewife abundances were routinely estimated in the inshore region of the zooplankton survey grid, I had a second source of alewife data to investigate the relationship between long-term changes in zooplankton and alewife abundance. Fourth, by using the same zooplankton data set and various estimates of long-term trends in alewife abundance, I was able to investigate how the source of the alewife data used in the analysis affected the outcome of the correlation analyses.

Zooplankton data were collected in the "offshore" region (20- to 40-m depth contours) of southeastern Lake Michigan as part of the Donald C. Cook nuclear power plant study (Evans et al. 1985). Zooplankton species considered for these analyses were *Diaptomus ashlandi*, *D. minutus*, *D. oregonensis*, *D. sicilis*, and *L. macrurus* as reported in Evans (1990), *D. retrocurva* and *D. galeata mendotae* as reported in Evans and Jude (1986), and *Epischura lacustris*, *B. longirostris*, and *Leptodora kindtii* (see Evans et al. 1985). Other species were not included in the analyses because they occurred too sporadically (i.e. too many zero values) to merit analysis. Summer mean abundances (July–August average) were calculated for each year of the 1972–81 period as described in Evans and Jude (1986) and Evans (1990). Mean species abundances then were correlated (Pearson's correlation) with four estimates of alewife abundance: lakewide census data for 1972–81 as presented in Eck and Wells (1987) and later in Evans (1990); lakewide census data for 1973–81; annual mean catches (1973–81) for the Cook study area as reported in Jude and Tesar (1985) and later in Evans (1990); and mean summer catches (1973–82) for the Cook study area as reported in Evans and Jude (1986).

Correlations also were calculated between phytoplankton abundances at the Chicago intake and alewife abundances for the five zooplankton–alewife comparisons (one for offshore of Grand Haven; four for offshore of the Cook plant) described above. Phytoplankton data are from Damann (1960) (1926–58), Vaughn (1970) (1959–69), Danforth and Ginsburg (1980) (1960–75), Makarewicz and Baybutt (1981) (1976–77), and E. P. Flanagan (Department of Water, Chicago, IL, pers. comm.) (1978–89). A second series of correlations were calculated between mean summer Secchi disc depth in the offshore region of the Cook monitoring area and the various estimates of long-term trends in alewife abundance. Secchi disc data are from Evans et al. (1985) and earlier cited reports.

Results

Long-Term Trends in Lakewide Alewife Abundances

Alewife (Fig. 1a) were extremely sparse in Lake Michigan in 1954 (relative abundance = 0.001), reached record abundances in 1966 (relative abundance = 1.0), and, following the spring 1967 dieoff, remained in depressed abundances in 1968 (relative abundance = 0.25). Alewife abundances in 1977, 1982, and 1987 (relative abundance = 0.23–0.29) were approximately equivalent to those observed in 1968. Abundances were substantially lower in 1984, 1985, and 1986 (relative abundance = 0.06–0.15) than in 1968 although higher than in 1954.

If alewife were the only fish species affecting zooplankton community structure, large-bodied zooplankton should have occurred in greatest abundances in 1954 and lowest abundances in 1966. The remaining years should have been years of intermediate abundances of large-bodied zooplankton. Moreover, because alewife abundances were some 3.5 (1987) to 16.7 (1984) times more abundant in 1966 than in later years, 1966 should be a year of strikingly low abundances of large-bodied zooplankton. The response of medium- and small-bodied zooplankton to changes in alewife abundance is less predictable. If alewife predation on the zooplankton community were moderate, reductions in the abundance of large-bodied grazers (*Daphnia* spp.), omnivores (*D. sicilis* and *L. macrurus*), and carnivores (*Mesocyclops edax* and *E. lacustris*) might have allowed medium- and small-bodied zooplankton to increase in abundance. If alewife predation were sufficiently intense, particularly in 1966, small- and medium-bodied zooplankton might also have been reduced in abundance; intense yellow perch (*Perca flavescens*) predation has been implicated in the marked reduction of all size categories of zooplankton in the inshore region over the summers of 1982–84 (Evans 1986).

Diaptomus Species Complex

Four species of *Diaptomus* typically are found in the offshore waters of Lake Michigan. *Diaptomus minutus*, an epilimnetic

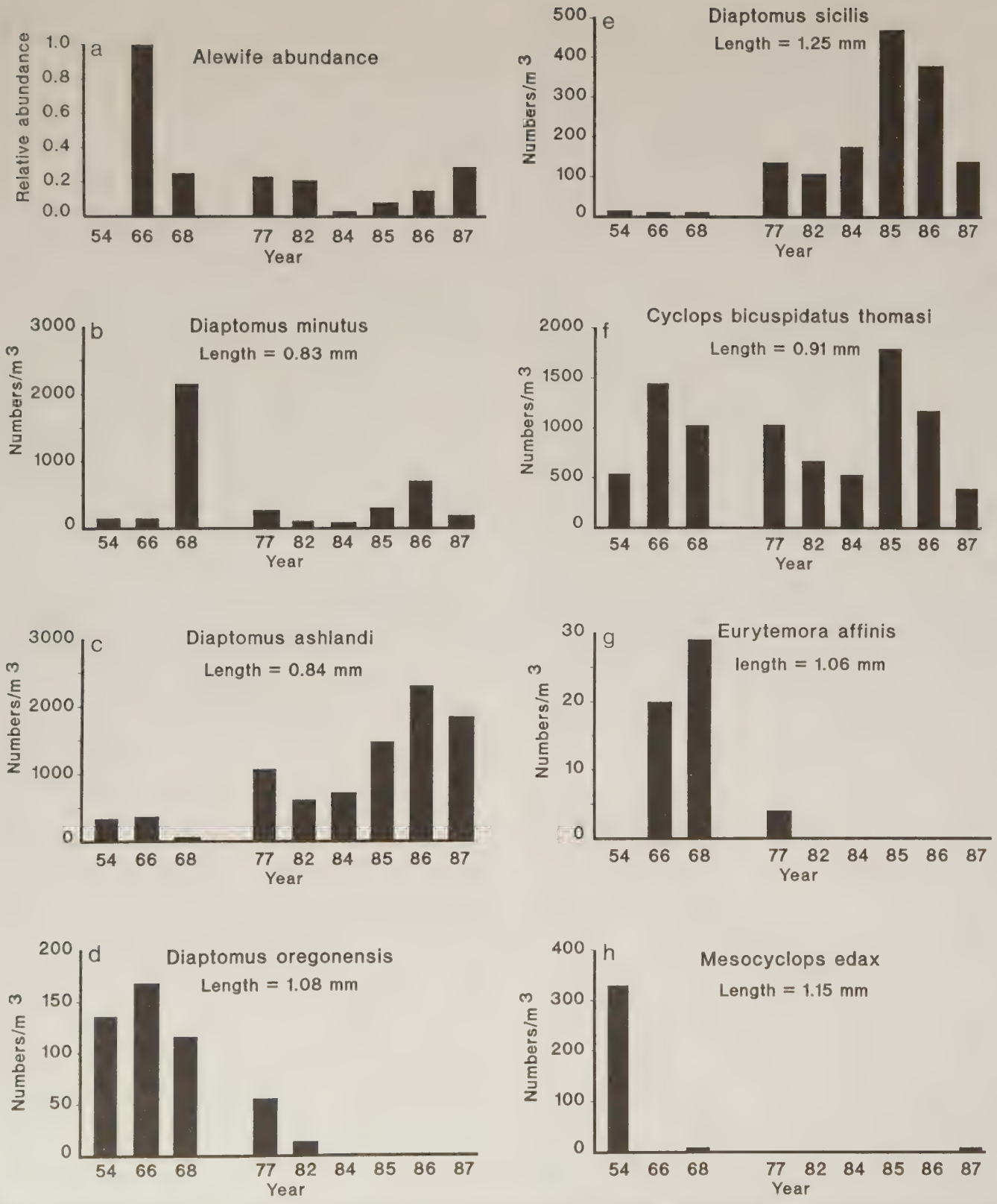

FIG. 1. (a) Relative variation in mean lakewide alewife abundances. Also shown are mean summer (July–August averages) abundances of (b) *D. minutus*, (c) *D. ashlandi*, (d) *D. oregonensis*, (e) *D. sicilis*, (f) *C. bicuspidatus thomasi*, (g) *Eutytemora affinis*, and (h) *M. edax* collected in the offshore region of Lake Michigan.

species, and *D. ashlandi*, a metalimnetic species, are the smallest with a 1954 mean length of 0.83 and 0.84 mm, respectively (Wells 1960, 1970). *Diaptomus oregonensis*, also an epilimnetic species, is the third largest with a 1954 mean length of 1.08 mm. *Diaptomus sicilis* is the largest species with a 1954 mean length of 1.25 mm (Wells 1970). Wells (1960) reported *D. sicilis* as preferring the upper layers (i.e. above 30 m) although it avoided the surface during bright daylight.

Diaptomus minutus occurred in approximately similar abundances (Fig. 1b) in summer 1954, when alewife were rare, as in summer 1966, during the height of the alewife population explosion. Maximum abundances were attained in 1968. Similarly, *D. ashlandi* occurred in similar abundances in 1954 and 1966: abundances were lower in 1968 (Fig. 1c). Moreover, *D. ashlandi* abundances were substantially higher over 1977–87 than during the summers considered by Wells. *Diaptomus oregonensis* occurred in maximum abundances in 1966 and in slightly lower abundances in 1954 and 1968. Abundances were substantially lower in later years (Fig. 1d). *Diaptomus sicilis* was very rare during Wells' study, occurring in average abundances of 8, 2, and 3/m^3 in 1954, 1966, and 1968, respectively (Fig. 1e). Abundances were substantially higher in later years. For the four *Diaptomus* spp., only the correlation ($r = -0.47$) between *D. sicilis* and alewife abundances was significant ($p = 0.05$) over the nine years considered (Table 1). The extremely low *D. sicilis* abundances in 1954 and 1968 were contrary to statistical model predictions.

Other Copepods

Wells (1970) reported that in summer 1954, *Cyclops bicuspidatus thomasi* had a mean length of 0.91 mm, while *M. edax* had a mean length of 1.15 mm. Both cyclopoids were reported to be epilimnetic (Wells 1960). For the years investigated by Wells (1970), *C. bicuspidatus thomasi* (Fig. 1f) was most abundant in 1966 and least abundant in 1954. However, *C. bicuspidatus thomasi* exhibited similarly strong interannual variability in abundance over 1977–87. The correlation between *C. bicuspidatus thomasi* and alewife abundances over the nine-year data set was weak and not significant (Table 1). *Mesocyclops edax* was detectable in the plankton only in the summers of 1954, 1968, and 1987 (Fig. 1h). The data set was insufficient for statistical analyses because of the large number of zero values for *M. edax* abundances.

Eurytemora affinis is an epilimnetic calanoid with a mean length of 1.06 mm in summer 1966 (Wells 1970). It is a brackish-water copepod, first being observed in Lake Michigan in 1964 (Robertson 1966). Abundances were higher in 1968 than in 1966 (Fig. 1g). With the exception of 1977, *E. affinis* abundances were below detection limits in later years. The data set was insufficient for statistical analyses because of the large number of zero values for *E. affinis* abundances. While not detected in offshore waters, *E. affinis* was a common constituent of the inshore-region summer zooplankton community over 1972–81 (Evans et al. 1985).

In summer 1954, *E. lacustris*, an epilimnetic calanoid, had a mean length of 1.70 mm (Wells 1960, 1970). In contrast with *M. edax*, *E. lacustris* was a relatively frequent constituent of the offshore summer zooplankton community over both 1954–68 and 1977–87 (Fig. 2a). For the period considered by Wells (1970), *E. lacustris* abundances were substantially lower in 1966 than in 1954 and 1968. Interannual variation in *E. lacustris* abundances over 1977–87 also was large. The correlation between *E. lacustris* and alewife abundance was not significant (Table 1) for the 9-yr data set.

In summer 1954, *L. macrurus* had a mean length of 2.45 mm (Wells 1970). For the period considered by Wells (1970), lowest *L. macrurus* abundances (Fig. 2b) were observed in 1966; highest abundances were observed in 1968. However, *L. macrurus* abundances were also highly variable over 1977–87. The correlation between *L. macrurus* and alewife abundances was not significant (Table 1).

In summer 1954, *S. calanoides* had a mean length of 2.89 mm (Wells 1960, 1970). This species was exceedingly rare over 1954–68 (Fig. 2c). Lowest abundances occurred in

TABLE 1. Correlation coefficients (Pearson product moment) between summer zooplankton abundances offshore of Grand Haven, Michigan, and the offshore region of southeastern Lake Michigan (Cook monitoring study) for various time intervals and fish sampling studies. Zooplankton at Grand Haven were collected in 1954, 1966, and 1968 (Wells 1970) and 1977, 1982, and 1984–87 (Evans, 1990). Alewife data were collected by the U.S. Fish and Wildlife Service (USFWS) during their autumn lakewide census studies. Alewife data collected by Jude and Tesar (1985) were from 5- to 10-m depth region of the Cook monitoring area. Chicago algal data are from Fig. 4. Secchi disc data are from the offshore region of the Cook plant. See text for additional explanation. **Significance level of 0.05; *significance level of 0.10.

		Cook Power Plant			
	Grand Haven 1954–87 USFWS	1972–81 USFWS	1973–81 USFWS	1973–81 Jude and Tesar (1985): annual	1973–81 Jude and Tesar (1985): summer
Copepods					
Cyclops b. thomasi	+0.13	+0.25	+0.11	+0.45	+0.16
Diaptomus ashlandi	−0.03	+0.63**	+0.59**	+0.25	+0.24
D. minutus	+0.07	+0.42	+0.34	+0.48*	+0.30
D. oregonensis	+0.32	0.00	−0.09	+0.55*	+0.46*
D. sicilis	−0.47*	−0.20	−0.06	−0.33	−0.57*
Epischura lacustris	−0.13	−0.76**	−0.77**	−0.77**	−0.69**
Limnocalanus macrurus	−0.28	−0.11	−0.05	−0.45	−0.51*
Cladocerans					
Daphnia g. mendotae	−0.60**	−0.58**	−0.96**	−0.27	+0.08
D. retrocurva	−0.45	+0.20	+0.18	+0.50*	+0.55*
D. pulicaria	−0.18	—	—	—	—
Bosmina longirostris	+0.48*	+0.52*	+0.46*	+0.52*	+0.28
Leptodora kindtii	−0.67**	+0.09	+0.28	+0.56*	+0.64**
Algae (Chicago)	+0.02	+0.65**	+0.63**	+0.90**	+0.64**
Secchi (Cook)	—	−0.20	−0.25	−0.65**	−0.41
Number of observations	9	10	9	9	9

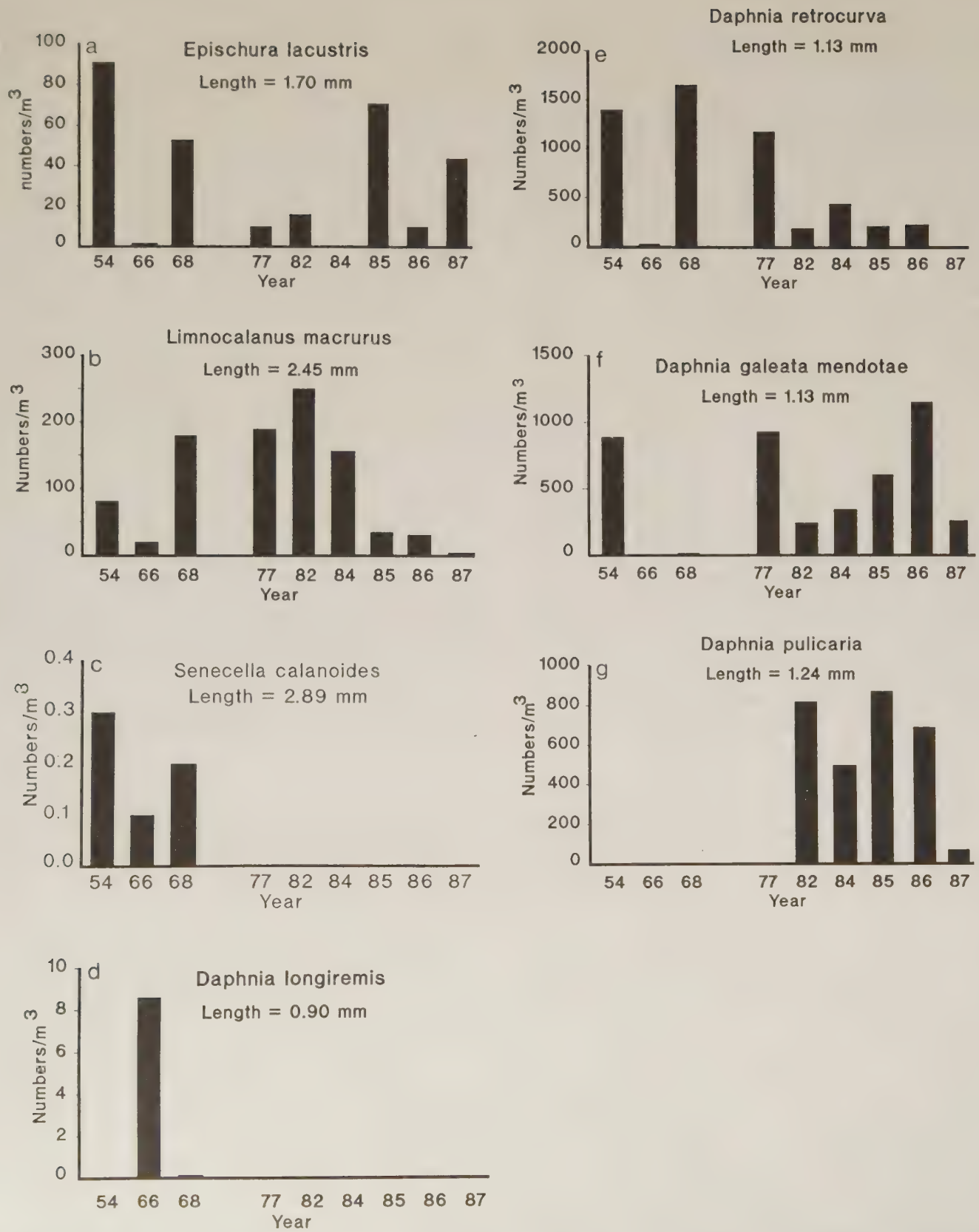

FIG. 2. Mean summer (July–August averages) abundances of (a) *E. lacustris*, (b) *L. macrurus*, (c) *S. calanoides*, (d) *D. longiremis*, (e) *D. retrocurva*, (f) *D. galeata mendotae*, and (g) *D. pulicaria* collected in the offshore region of Lake Michigan.

1966, at the height of the alewife population explosion. *Senecella calanoides* was not detected in the 0-to 40-m plankton tows in later years. Nor was it detected in deeper plankton tows made from 95 to 40 m in July and August 1977 (M. S. Evans, unpubl. data). *Senecella calanoides* also was not detected during the long-term (1972–82) monitoring study of zooplankton populations in southern Lake Michigan (Evans et al. 1985; M. S. Evans, unpubl. data). However, low numbers ($<5/m^3$) of

immature and adult *S. calanoides* copepodites were observed in 95- to 0-m tows made in 1985, 1986, and 1987 in the region offshore of Grand Haven (Lehman 1988).

Daphnia Species Assemblage

Only two species of *Daphnia* (Fig. 2e, 2f) were commonly observed over 1954–68: *D. retrocurva* with a mean length of 1.13 mm in 1954, 0.94 in 1966, and 1.00 in 1968 and *D. galeata mendotae* with a mean length of 1.13 mm in 1954. A third species, *D. longiremis* (Fig. 2d), was observed only in 1966 and 1968; its mean length in 1966 was 0.90 mm (Wells 1960). *Daphnia retrocurva* and *D. galeata mendotae* inhabit the epilimnion (Wells 1960), while *D. longiremis* prefers hypolimnetic waters (Brooks 1957).

Daphnia retrocurva occurred in sharply lower abundances (Fig. 2a) in 1966 than 1954; abundances were somewhat greater in 1968 than in 1954. *Daphnia retrocurva* exhibited a large amount of interannual variability over 1977-87; it was not observed in July–August 1987. *Daphnia retrocurva* and alewife abundances were negatively correlated ($r = -0.45$) although the correlation was not significant (Table 1). Similarly, *D. galeata mendotae* abundances were relatively high in 1954 but were below detection limits in 1966 and 1968 (Fig. 2f). *Daphnia galeata mendotae* exhibted a large amount of interannual variability over 1977–87 and was always readily detected in the plankton. There was a strong and significant negative correlation between *D. galeata mendotae* and alewife abundances ($r = -0.60$).

A fourth daphnid, *D. pulicaria*, is the largest species attaining body lengths of over 3 mm (Evans 1985; Schulze and Brooks 1987). In contrast, Wells (1970) reported maximum body lengths of 1.80 mm for *D. galeata mendotae* and 1.71 mm for *D. retrocurva*. *Daphnia pulicaria* was not detected (Fig. 2g) in the zooplankton in summer 1954; a few *D. schølderi* (probably *D. pulicaria*) were collected in 1966 and 1968 although abundances were not given. *Daphnia pulicaria* was occasionally detected in southeastern Lake Michigan over 1978–81 but did not become abundant until 1982 (Evans and Jude 1986). Abundances remained relatively constant over 1982–86 and then decreased sharply in 1987. The correlation between *D. pulicaria* and alewife abundance was weak ($r = -0.18$) and not statistically significant (Table 1). The analysis was rerun, excluding 1954, 1966, and 1968, years in which *D. pulicaria* was not detected in the plankton. The correlation was strengthened ($r = -0.54$) but was not statistically significant even at $p = 0.10$.

Other Cladocerans

Six other small-bodied (0.48–0.85 mm) cladoceran species were detected in the plankton at various times over the two study periods (Fig. 3a–3f). *Bosmina longirostris* was consistently observed in the plankton over the study period. *Bosmina longirostris* was more abundant in 1966 than in 1954 and 1968. Abundances generally were greater over 1977–87 than during Wells' study. There was a moderately strong negative correlation ($r = -0.48$) between *B. longirostris* and alewife abundances although this correlation was significant only at the $p = 0.10$ level (Table 1). *Eubosmina coregoni*, *Ceriodaphnia* sp., *Polyphemus pediculus*, *Holopedium gibberum*, and *Diaphanosoma* sp. were extremely sporadic in occurrence; abundances were below detection limits in most years. In contrast, *L. kindtii* (1954 body length approximately 5 mm) was detected in most years (Fig. 3g). Interannual variability in abundance was large over 1954, 1966, and 1968; minimum abundance occurred in 1966. Interannual variability in abundance also was large over 1977–87. There was a strong and significant negative correlation ($r = -0.67$) between *L. kindtii* and alewife abundances.

Zooplankton and Alewife Abundance Correlations: Southeastern Lake Michigan

The offshore region of southeastern Lake Michigan provides a second location in which to test the hypothesis that long-term changes in zooplankton abundance can be directly attributed to long-term changes in alewife abundance (Table 1). The first series of analyses was based on the correlation of zooplankton abundance with lakewide estimates of alewife abundance; the period analyzed was 1972–81. Correlations were significant ($p = 0.05$) for *D. ashlandi* ($r = +0.63$), *E. lacustris* ($r = -0.76$), and *D. galeata mendotae* ($r = -0.58$). The correlation between *B. longirostris* and alewife abundances ($r = +0.52$) was significant at $p = 0.10$. Similar correlation coefficients were obtained when 1972 was removed from the data analyses. This year was removed from the analyses to allow for a comparison of the correlation coefficients based on lakewide estimates of alewife abundance and the two estimates (annual, summer) of long-term trends in alewife abundance in the inshore region of southeastern Lake Michigan.

Correlations between zooplankton and annual alewife catches in the inshore region of southeastern Michigan were significant at $p = 0.05$ for *E. lacustris* ($r = -0.77$) and significant at $p = 0.10$ for *B. longirostris* ($r = +0.52$); correlation coefficients were similar to those calculated based on lakewide alewife abundance estimates. Additional correlations which were significant at $p = 0.10$ were *D. minutus* ($r = +0.48$), *D. oregonensis* ($r = +0.55$), *D. retrocurva* ($r = +0.50$), and *L. kindtii* ($r = +0.56$). While alewife and *L. kindtii* abundances were positively correlated in this analysis, abundances were negatively correlated when based on zooplankton collections made offshore of Grand Haven (Table 1).

Correlations between zooplankton and summer alewife catches in the inshore region of southeastern Lake Michigan were significant at $p = 0.05$ for *E. lacustris* ($r = -0.69$) and *L. kindtii* ($r = +0.64$). Additional correlations which were significant at $p = 0.10$ were *D. retrocurva* ($r = +0.55$), *D. oregonensis* ($r = +0.46$), *D. sicilis* ($r = -0.57$), and *L. macrurus* ($r = -0.51$). Correlations between zooplankton and inshore region alewife abundance were similar for most zooplankton taxa when based on annual or summer alewife catches. The notable exceptions were *C. bicuspidatus thomasi*, *D. sicilis*, *D. galeata mendotae*, and *B. longirostris*.

Phytoplankton, Alewife, and Secchi Disc Depth Correlations

The correlation between algal abundances at the Chicago intake (Fig. 4) and lakewide estimates of alewife abundances was low ($r = +0.02$) and not significant for the 9-yr data set considered (1954, 1966, 1968, 1977, 1982, 1984–87) in the reexamination of Wells' (1970) original study (Table 1). The analysis was rerun using all years in the 1954–87 data set (Fig. 4); the correlation remained low ($r = +0.01$) and nonsignificant.

A second series of correlation analyses was performed for the 1972–81 period and lakewide alewife abundance estimates (Fig. 4; Table 1). In this instance, algal abundances were significantly correlated ($r = +0.65$) with alewife abundances. Similar correlations were obtained when the analysis was based on the period 1973–81 and both the lakewide estimates of ale-

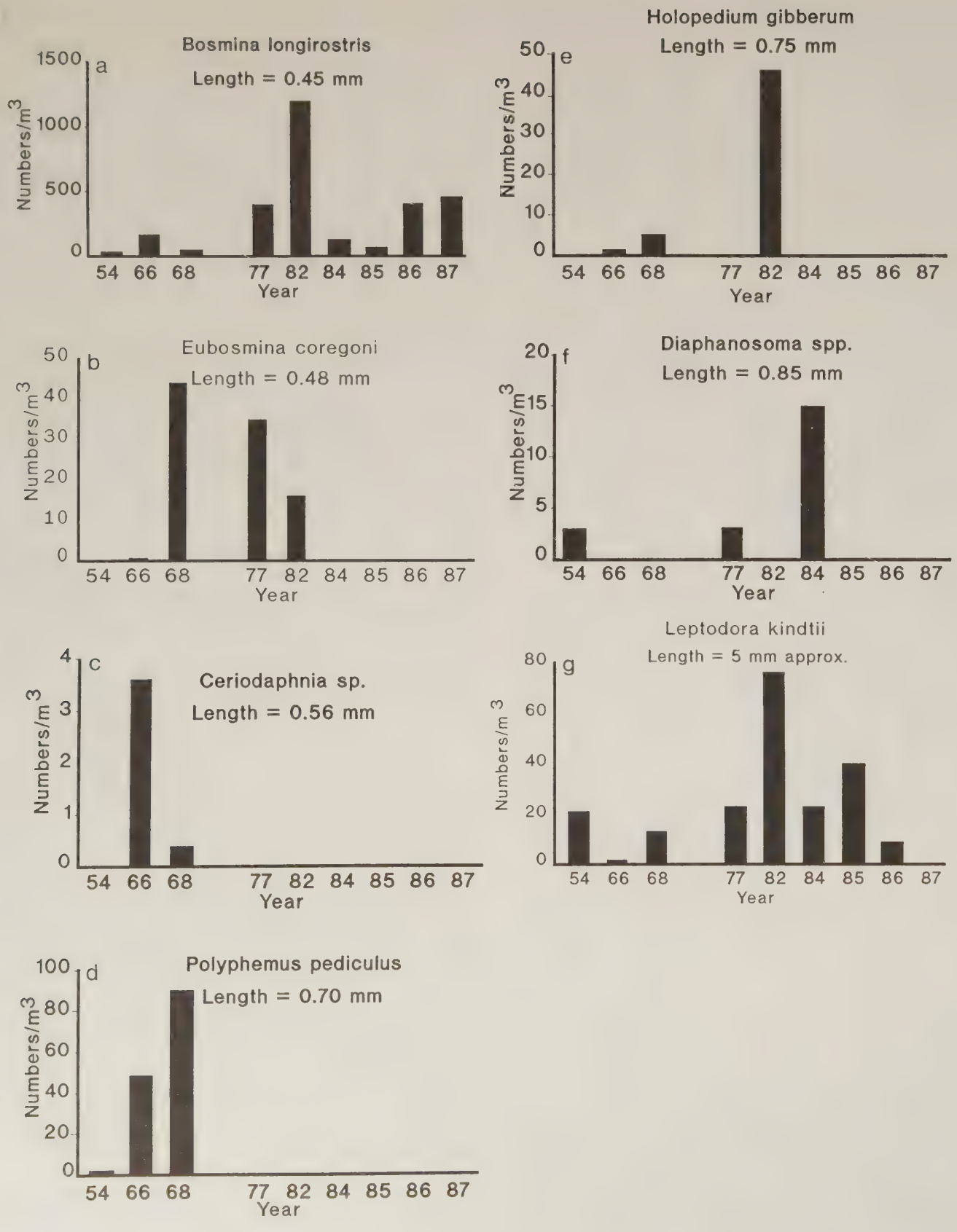

FIG. 3. Mean summer (July–August averages) abundances of (a) *B. longirostris*, (b) *E. coregoni*, (c) *Ceriodaphnia* sp., (d) *P. pediculus*, (e) *H. gibberum*, (f) *Diaphanosoma* sp., and (g) *L. kindtii* collected in the offshore region of Lake Michigan.

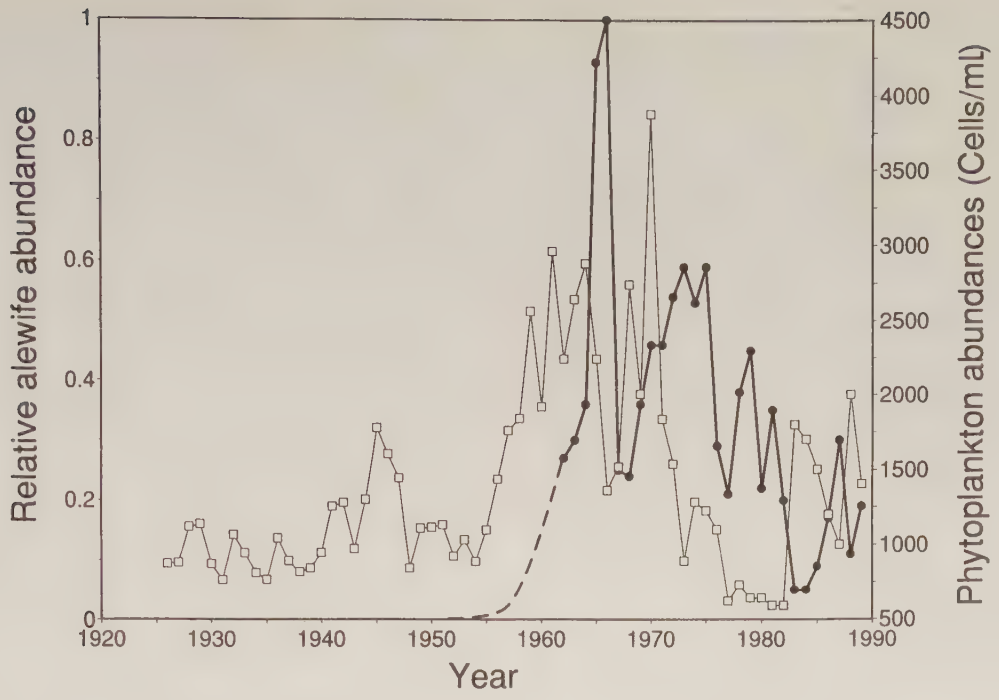

FIG. 4. Long-term trends in alewife (closed circles) and phytoplankton (open squares) abundances in Lake Michigan. Alewife data are modified from fig. 1 in Wells (1985) and fig. 1 in Brown (1990), with permission of the authors. Phytoplankton data are from the Chicago water filtration plant as described in text.

wife abundance and summer estimates of alewife abundance in the inshore region of southeastern Lake Michigan. The highest coefficient was obtained when algal abundances at the Chicago intake were correlated with annual estimates of alewife abundance in the inshore region of southeastern Lake Michigan.

Summer Secchi disc depths in the offshore region of southeastern Lake Michigan were significantly correlated ($r = -0.65$) with annual estimates of alewife abundance in the inshore region of the Cook study area (Table 1). Other correlations, while negative, were substantially lower and not statistically significant.

Discussion

According to the top-down hypothesis as applied to Lake Michigan, long-term increases (from the late 1950s to early 1960s) and decreases (late 1960s to early 1970s) in phytoplankton abundance can be attributed to an alewife-mediated effect on zooplankton grazing pressure (Brooks et al. 1984; Edgington 1984, 1986). The validity of this hypothesis is dependent on a number of assumptions which are explored in the following sections.

Long-Term Trends in Zooplankton and Alewife Abundance

The top-down argument is strongly based on purported differences in zooplankton species abundance in the summers of 1954, 1966, and 1968 which were attributed to changes in alewife predation (Wells 1970). Based on the results of Wells' (1970) study, researchers have inferred changes in relative grazing pressure on the phytoplankton community based on estimated changes in alewife abundance (Brooks et al. 1984; Edgington 1984, 1986).

With increased environmental monitoring and research, we have a greater awareness of the large interannual variability in the abundances of most zooplankton (Evans and Sell 1983; Evans et al. 1985; Pedros-Alio and Brock 1985). Consequently, relatively long-term data sets are required to detect trends in zooplankton species abundance (Evans and Jude 1986; Evans 1990). Moreover, while some trends in zooplankton species abundance can be related to factors such as changes in alewife abundance, other trends require alternative explanation: long-term trends in bloater (*Coregonus hoyi*) and yellow perch abundance, invasion of zoooplankton species new to Lake Michigan, invertebrate predation, and competition between related species (Evans 1986, 1990; Evans and Jude 1986; Lehman 1987, 1991; McDonald et al. 1990). Other long-term trends in zooplankton species abundance remain unexplained (Evans 1990). With this increased understanding of interannual variability in zooplankton species abundances over the 1970s and 1980s, it is now possible to reevaluate Wells' (1970) original study to investigate the possible effect of changes in alewife abundance on zooplankton abundance during earlier time periods.

In his original study, Wells (1970) observed many differences in zooplankton species abundances between the summers of 1954, 1966, and 1968. Large-bodied species such as *L. kindtii*, *D. galeata mendotae*, *D. retrocurva*, *L. macrurus*, *E. lacustris*, *D. sicilis*, and *M. edax* "declined sharply in abundance between 1954 and 1966," while medium-bodied or small species such as *D. longiremis*, *H. gibberum*, *P. pediculus*, *B. longirostris*, *Eubosmina coregoni*, *Ceriodaphnia* sp., *C. bicuspidatus thomasi*, *C. vernalis*, and *D. ashlandi* increased in abundance. Moreover, Wells (1970) stated that the "evidence is strong that the changes were due to selective predation by alewives," i.e. size-selective predation on the largest zooplankton increased with the 1954 to 1966 alewife population explosion. Following the spring 1967 die-off, most zooplankton species apparently had returned to their approximate 1954

abundance with the exception of *D. galeata mendotae* and *M. edax* (Wells 1970). While these interpretations were appropriate in 1970, they appear to be less appropriate today given our newer understanding of the Lake Michigan ecosystem.

Wells' (1970) study did show that the large-bodied *D. silicis*, *L. macrurus*, *M. edax*, *E. lacustris*, *D. galeata mendotae*, *D. retrocurva*, and *L. kindtii* were less abundant in 1966 than in 1954 and 1968; this may suggest that alewife had a major effect in reducing the abundance of these large-bodied zooplankton. However, three of these large-bodied zooplankton, *L. macrurus*, *E. lacustris*, and *D. retrocurva*, were more abundant in 1968 than in 1954, suggesting that factors in addition to alewife abundance must account for differences in their abundance between the summers of 1954, 1966, and 1968. Only *D. sicilis*, *E. lacustris*, *M. edax*, and *D. galeata mendotae* were most abundant in 1954 and least abundant in 1966.

According to Wells (1970), the small- and medium-bodied *D. longiremis*, *H. gibberum*, *P. pediculus*, *B. longirostris*, *E. coregoni*, *Ceriodaphnia* sp., *C. bicuspidatus thomasi*, *C. vernalis*, and *D. ashlandi* increased in abundance between 1954 and 1966. However, only *B. longirostris*, *D. longiremis*, and *Ceriodaphnia* sp. were most abundant in 1966 and least abundant in1954. The small-bodied *D. minutus* was least abundant in 1966.

Many of the taxa reported by Wells (1970) to have been strongly affected by the alewife population explosion varied as much in abundance between 1954, 1966, and 1968 as between 1977, 1982, and 1984–87. For example, *C. bicuspidatus thomasi* exhibited a 2.7-fold difference in mean abundance in the three summers investigated by Wells (1970) and a 4.5-fold difference between 1977, 1982, and 1986–87. Similar differences between the two study periods were observed for *D. ashlandi* (4.6 versus 3.8), *D. minutus* (14.4 versus 9.5), *D. sicilis* (4.0 versus 4.4), and *L. kindtii* (7.0 versus 8.4). Other taxa exhibited more substantial variations between the two study periods: *D. oregonensis* (1.5 versus >55), *L. macrurus* (9.0 versus 31.4), *D. galeata mendotae* (445 versus 4.7), *D. retrocurva* (34.4 versus 5.5), and *B. longirostris* (5.1 versus 16.7). Only *D. galeata mendotae* and *D. retrocurva* exhibited greater variation in abundance over the former (1954, 1966, and 1968) than the latter (1977, 1982, and 1984–87) period; this greater variation in the abundance of these taxa is consistent with the greater variation in alewife abundance in the former period.

The correlation between zooplankton and alewife abundance across the two study periods (1954, 1966, 1968; 1977, 1982, and 1984–87) was significant ($p = 0.05$) only for the large-bodied *D. galeata mendotae* and *L. kindtii*. If a lower significance level is accepted ($p = 0.10$), then *D. sicilis* and *B. longirostris* abundances were significantly correlated with alewife abundances over the two study periods. However, because *D. sicilis* was substantially more abundant in 1977 and the 1980s than in 1954 and 1968, alewife predation alone cannot account for the low abundances of this zooplankton species observed during Wells' (1970) study. Similarly, *B longirostris* was substantially more abundant in 1977, 1982, 1986, and 1987 than in 1966; differences in the mesh of the plankton net used during these two studies might account for some (or all) of these differences. The correlation between *D. retrocurva* and alewife abundance ($r = -0.45$), while high, was not statistically significant even at $p = 0.10$.

In summary, the reexamination of Wells' (1970) study suggests that the effects of increased alewife abundances in 1966 and 1968 on the zooplankton community are less evident than originally proposed. Only *D. galeata mendotae* abundances appeared to be consistently related to increases and decreases in alewife abundance. *D. pulicaria*, the zooplankton grazer implicated in the increase in water clarity and decrease in chlorophyll concentrations during the summers of 1983 and 1984 (Scavia et al. 1986), was not detected during Wells' (1970) study. Consequently, the abundance of this species could not be related to fluctuations in alewife abundance over the 9-yr data set which spanned 1954–87.

Other Factors Affecting Zooplankton Abundances between 1954, 1966, and 1968

One of the most interesting aspects of Wells' (1970) study was the strong decrease in *D. galeata mendotae* and *D. retrocurva* abundances in 1966 relative to their 1954 abundances; *D. galeata mendotae* abundances were 445 times lower and *D. retrocurva* abundances were 34 times lower in 1966 than in 1954. These strong decreases have been related to intense alewife predation. What is particularly interesting about these data is that other large-bodied zooplankton did not show similar magnitudes of decreased abundances. In contrast, small-, medium-, and large-bodied zooplankton apparently were dramatically reduced in abundance in the inshore region of Lake Michigan by increased yellow perch predation (Evans 1986). A second interesting aspect of Wells' (1970) study was the apparent slow recovery time of *D. galeata mendotae* from the apparently intense predation in 1966 to the lower predation in 1968. Cladocerans have relatively rapid reproductive rates and hence should recover faster from population lows than the more slowly reproducing copepods (Allen 1976). Thus, *D. retrocurva* apparently had recovered by 1968; however, abundances of the slightly larger *D. galeata mendotae* remained exceedingly low. This is puzzling because abundances of the more slowly reproducing *L. macrurus*, *D. sicilis*, and *E. lacustris* apparently had recovered by 1968. Moreover, various comparisons of Wells' (1970) data with more recent data have revealed a number of inconsistencies in the argument that differences in zooplankton abundances between 1954, 1966, and 1968 can be attributable solely to changes in alewife predation. Accordingly, the causal factor(s) for the exceedingly low *Daphnia* abundances in summer 1966 is not fully understood and remains open to some speculation.

Several researchers, in addition to Wells (1970), noted that 1966 was an unusual year. Spring was relatively warm resulting in rapid thermal stratification and a relatively cold hypolimnion (Ayers and Strong 1967). Similarly, thermal data presented in Wells (1970, table 2) indicate that surface waters were substantially warmer (22.3 versus 16.8° C), while the hypolimnion was colder (4.9–5.6 versus 5.6–6.6° C) over late June to early July 1966 than in 1954. The hypolimnion continued to remain relatively cold through early August 1966 (4.6–6.1° C) when compared with temperatures recorded in 1954 (5.3–6.5° C) and 1968 (5.0–8.1° C). These lower hypolimnetic temperatures may have been a contributing factor to the fact that *D. longiremis* was a significant component of the daphnid assemblage in summer 1966 although not in 1954 and 1968. A cooling trend apparently occurred later in summer 1966; epilimnetic temperatures were lower in early August 1966 (14.4–18.3° C) than in 1954 (20.5–20.6° C) and 1968 (18.8–20.6° C). Overall, 1966 was the coolest (mean air temperature 7.15 versus 7.59° C in 1968 and 7.88° C in 1954) and driest (mean annual precipitation 746.6 versus 868.8 mm in 1968 and 942.2 mm in 1954) of the three years considered by Wells. Lake level also was the lowest (175.9 m above sea level versus 176.2 m in 1968 and

176.6 m in 1954) (F. Quinn, Great Lakes Environmental Research Laboratory, Ann Arbor, MI, pers. comm.).

Differences in meteorological conditions between the summers of 1954, 1966, and 1968 may have effected some of the interannual variability in zooplankton abundance as observed by Wells (1970). Early thermal stratification in spring 1966 may have resulted in a rapid depletion of nutrients from the epilimnion; phytoplankton standing stocks may have been reduced relative to years in which stratification took longer to develop. These lower algal standing stocks during late spring and early summer 1966 may have limited *Daphnia* reproduction. The cooling trend which occurred later in July 1966 would have resulted in an infusion of nutrient-rich hypolimnetic waters into the nutrient-poor epilimnion. While this nutrient infusion should have stimulated epilimnetic phytoplankton production, *Daphnia* abundances may have been too reduced to exhibit a strong recovery; alternatively, Wells' (1970) study did not extend sufficiently far into the mid- to late summer to observe this population pulse. However, these speculations do not explain why *D. galeata mendotae* and *D. retrocurva* would have been the only zooplankton species adversely affected by meteorological events in spring–summer 1966. Nor can these speculations explain why the *D. galeata mendotae* population had not recovered by 1968.

Other researchers also noted interesting phenomena in 1966. Beginning in mid- to late August, milky water and reduced Secchi disc depths were noted at a number of stations in the offshore region of the lake (Ayers et al. 1967). Milky water was attributed to calcium carbonate precipitation; such precipitation occurs when algal productivity is high (Wetzel 1975).Moreover, in August and September 1966, large numbers of dead mysids (both immatures and adults) were collected in plankton tows made in several regions of the lake (Ayers et al. 1967); the causal factor(s) for this unusual occurrence could not be identified. The following winter, the massive die-off of alewife occurred followed by an even larger die-off in June–July 1967. This die-off has been attributed to poor condition resulting from limited food resources (Brown 1972). With the possible exceptions of *D. galeata mendotae* and *D. retrocurva*, there is little direct evidence that alewife strongly reduced their offshore zooplankton food resources in summer 1966.

Considerations of Zooplankton Grazing Pressure

According to the top-down hypothesis, changes in zooplankton grazing pressure were the primary factor affecting increases and decreases in the phytoplankton abundances over the late 1950s and 1960s (Brooks et al. 1984; Edgington 1984, 1986). However, zooplankton grazing pressure on the phytoplankton community in the summers of 1954, 1966, and 1968 was not investigated as part of this hypothesis.

Zooplankton grazing pressure is affected by a number of factors including the abundance and composition of the algal community (Wetzel 1975). Moreover, grazing pressure varies with the zooplankton species considered and the developmental stage of that species (Wetzel 1975). Adult *Diaptomus* and *Limnocalanus* filtration rates on phytoplankton species have been reported to range from 1 to 3 $mL \cdot animal^{-1} \cdot d^{-1}$; small-bodied cladocerans (*Ceriodaphnia*, *Bosmina*, *Diaphanosoma*, and *Chydorus*) have reported rates of some 0.2–5 $mL \cdot animal^{-1} \cdot d^{-1}$ (Wetzel 1975). Accordingly, copepods and small-bodied cladocerans, because of their low filtration rates, cannot exert intense grazing pressure on the phytoplankton community unless these zooplankton species are very abundant. Such was not the case in Wells' (1970) study where species abundances (0–40 m) generally ranged from 10 to 1000/m^3 or 0.01 to 1.0/L. Thus, the theoretical filtering rate for most zooplankton species (with the exception of *Daphnia*) was 0.02–5.0 $mL \cdot L^{-1} \cdot d^{-1}$. If it is assumed that all zooplankton (with the exception of *Daphnia*) shown in Figs. 1-3 had an average filtering rate of 2 $mL \cdot individual^{-1} \cdot d^{-1}$, the mean filtering rates (millilitres per litre per day) for the nine summers of study were 3.5 in 1954, 4.8 in 1966, 7.8 in 1968, 6.5 in 1977, 6.3 in 1982, 3.7 in 1984, 8.5 in 1985,10.1 in 1986, and 6.2 in 1987. These rates are conservative because Wells (1970) did not enumerate nauplii and immature copeodies; moreover, his nets may have undersampled small-bodied cladocerans. Nevertheless, these estimates are rigorous enough to support the contention that the grazing rates of most zooplankton species were low. Moreover, 1954 was not a summer of especially high filtration rates.

Daphnia, especially large-bodied forms, have relatively high grazing rates (Burns and Rigler 1967; Wetzel 1975). However, there is little evidence that large-bodied *Daphnia* exerted heavy grazing pressure on the phytoplankton community over the period considered by Wells (1970). The large-bodied *D. pulicaria*, which has been attributed to having caused high water clarity in summer offshore waters in 1983 and 1984 (Scavia et al. 1986), was not observed by Wells (1970) in 1954. Thus, this species cannot be implicated in the relatively low phytoplankton abundances in 1954 and, by implication, earlier years. *Daphnia* spp. (*D. galeata mendotae* and *D. retrocurva*) which were present in 1954 were relatively small cladocerans with mean lengths of some 1.13 mm (Wells 1970). Such small-bodied *Daphia* have relatively low filtration rates. A *Daphnia* with a mean length of 1.13 mm has an estimated filtering rate of 10.8 $mL \cdot animal^{-1} \cdot d^{-1}$; this estimate is based on the length versus filtering rate regression developed by Burns and Rigler (1967). In summer 1954, *Daphnia* spp. densities averaged 2300/m^3 (Wells 1970; this study, Fig. 2). Thus, the *Daphnia* assemblage filtering rate was 24.8 $mL \cdot L^{-1} \cdot d^{-1}$. At this rate, the *Daphnia* assemblage would require 40.4 d to clear the water column (0–40 m). If it is assumed that all the *Daphnia* were confined to the upper 10 m of the water column, the *Daphnia* assemblage filtering rate would increase to 99.2 $mL \cdot L^{-1} \cdot d^{-1}$ and the assemblage would have required 10.1 d to clear the water column. In contrast, Scavia et al. (1986) estimated that the *Daphnia*-dominated zooplankton community in August 1983 had a filtering rate of some 560.1 mL/d and hence required only 1.8 d to clear the epilimnion.

Daphnia spp. filtering rates for the nine summers of investigation were calculated based on the length versus filtering rate regression of Burns and Rigler (1967), the mean length of *Daphnia* as provided by Wells (1970), and abundances shown in Fig. 2. For years in which *D. retrocurva* and *D. galeata mendotae* lengths were not given, the mean species length for 1954 was used. A mean length of 1.28 mm (Schulze and Brooks 1987) was used for *D. pulicaria*. It also was assumed that all the *Daphnia* were concentrated in the upper 10 m of the water column. According to these calculations, the mean *Daphnia* spp. filtering rates (millilitres per litre per day) for the nine summers of study were 98.6 in 1954, 1.4 in 1966, 49.2 in 1968, 90.8 in 1977, 70.2 in 1982, 64.9 in 1984, 90.1 in 1985, 102.9 in 1986, and 15.8 in 1987. These rates are relatively low, with the rate for 1954 similar to that observed during 1977, 1985, and 1986. These results suggest that *Daphnia* grazing rates on the algal community were not high during most of the summers of study. Excluding 1966, algal turnover rate due to zooplankton grazing ranged from 9.8 to 63.3 d. In comparison, algal

turnover rates due to growth were estimated at 2.5–10 d for the summers of 1983 and 1984 (Scavia et al. 1988).

Inshore–Offshore Differences in Ecosystem Structure

The top-down argument that long-term trends in phytoplankton abundance are a consequence of long-term trends in alewife abundance is based on the comparison of population trends for phytoplankton in the inshore region (intake sampling) and hypothetical zooplankton trends for the offshore region. However, zooplankton community structure differs between the inshore and offshore regions (Evans et al. 1979). Moreover, the inshore and offshore regions exhibited quite different responses to long-term declines in alewife abundance (Evans and Jude 1986; Evans 1986, 1990) over the 1972–84 period. In contrast with the offshore region, large-bodied zooplankton such as *D. pulicaria* did not increase markedly in abundance in the inshore region with the alewife population decline. Moreover, zooplankton abundances apparently were decimated in the inshore region during the summers of 1982–84 by the strong increase in yellow perch abundances. Thus, Wells, (1970) study, while providing useful insight into offshore zooplankton community structure in the summers of 1954,1966, and 1968, provides little insight into zooplankton community structure in the inshore region over the same period. Therefore, long-term lakewide trends in alewife abundance cannot be linked (through zooplankton grazers) with long-term trends in phytoplankton abundances in the inshore region of southwestern Lake Michigan.

A second difficulty with linking long-term trends in phytoplankton abundance at a particular inshore region intake and lakewide trends in alewife abundance is associated with the fact that lakewide trends in alewife abundance may differ at the regional level. When alewife first invaded Lake Michigan from Lake Huron, the population increase proceeded from the north to the south (Wells and McLain 1973). There is also some evidence that the recent decline in alewife abundance in Lake Michigan occurred 5–6 yr earlier in southeastern Lake Michigan than lakewide (Evans 1990). These regional differences in alewife abundance trends can affect the correlations between alewife and zooplankton abundances. Correlations which are significant when lakewide autumn abundance estimates are used change and may become insignificant when regional data are used; other correlations may become significant (Table 1). Similarly, the correlation between alewife and photoplankton abundances is affected by the region from which alewife abundance estimates were determined (Table 1).

Phytoplankton abundances also vary regionally. Of particular relevance to this paper are the substantially greater abundances of phytoplankton at the Chicago than at the Milwaukee intake over the 1950s and 1960s (Edgington 1984, 1986). While algal abundances at Chicago exceeded 2000 cells/mL (annual average) six times during this period (Fig. 4), abundances at Milwaukee approached (or slightly exceeded) 1000 cells/mL on only three occasions. Moreover, algal abundances at Milwaukee were similar during the late 1940s to those observed during the early to mid-1960s. In contrast, algal abundances at Chicago were substantially lower during the latter than the former period (Fig. 4). Thus, the link between long-term trends in phytoplankton abundance at the Chicago intake and differences in zooplankton abundance in the region offshore of Grand Haven (Fig. 1–3) is unclear.

Correspondence between Trends in Alewife and Phytoplankton Abundance

Another weakness in the linking of long-term trends in phytoplankton and alewife abundance is the relative poor correspondence between the two time trends (Fig. 4). For example, phytoplankton abundances increased through the late 1950s and levelled off through the early 1960s; however, alewife abundances continued to increase markedly into the early 1960s reaching maximum abundances in 1966. Alewife abundances remained low for 2 yr following the spring 1967 population crash but then increased through the late 1960s to the mid-1970s. However, phytoplankton populations continued to decline through the mid-1970s approaching levels observed during the late 1920s to mid-1950s. Moreover, although the top-down argument hypothesizes that years of high alewife abundance would also be years of high phytoplankton abundance, such was not always observed. The most notable such year was 1966, a year of record high alewife abundances and of low phytoplankton abundances. Because *Daphnia* were exceedingly rare in offshore waters in summer 1966 (Wells 1970; this study, Fig. 2) and presumably were rare in inshore waters, it is unlikely that these low algal abundances were due to grazing. Rather, as previously discussed, it is possible that low algal abundances were an important factor in reducing *Daphnia* abundances in summer 1966. Phytoplankton abundances also were relatively low in 1967, a year of low alewife abundances and, according to inferences by Wells (1970), low *Daphnia* abundances.

Close examination of phytoplankton and alewife abundance trends during the late 1970s and the 1980s (Fig. 4) reveal other weaknesses in the top-down argument. Phytoplankon abundances reached record lows in the late 1970s and early 1980s although alewife remained relatively abundant and *D. pulicaria* rare during these periods. Phytoplankton abundances increased in 1983 and remained relatively high (in comparison with values observed in the late 1970s and early 1980s) in 1984. These high phytoplankton abundances in 1983 and 1984 occurred even though the large-bodied *D. pulicaria* was a major component of the summer offshore zooplankton assemblage in these years (Scavia et al. 1986). Phytoplankton abundances declined over 1985–87 (Fig. 4) although alewife abundances increased and *D. pulicaria* decreased in dominance (Dorazio et al. 1987).

The correlation between phytoplankton and lakewide alewife abundances (Fig. 4) was exceedingly low and not significant ($p = 0.05$) when either the entire 1954–88 data set was considered or when comparisons were limited to the 9 yr of zooplankton study (Grand Haven) within this data set. In contrast, significant correlations were obtained for the 1972–81 and 1973–81 comparisons; such comparisons were based on lakewide and regional (inshore region of southeastern Lake Michigan) estimates of alewife abundance. The reasons for this were not directly investigated but, as discussed above, cannot be attributed to intense grazing by large-bodied cladocerans such as *D. pulicaria* and *D. galeata mendotae*. Alternative hypotheses (i.e. the bottom-up hypothesis) are cited in the introduction.

Considerations of Phytoplankton Abundance Estimates

Long-term trends in phytoplankton abundances as used in the top-down argument are based on mean annual abundances of phytoplankton. However, phytoplankton are highly seasonal in abundance. In Lake Michigan, phytoplankton reach their highest densities in early spring and secondarily in late autumn. Thus, long-term trends in annual phytoplankton abundance are strongly weighted by events occurring during these two seasons. This argument is clearly supported by a study conducted at the Chicago filtration plant (Danforth and Ginsburg 1980) comparing mean annual phytoplankton abundances over 1972–

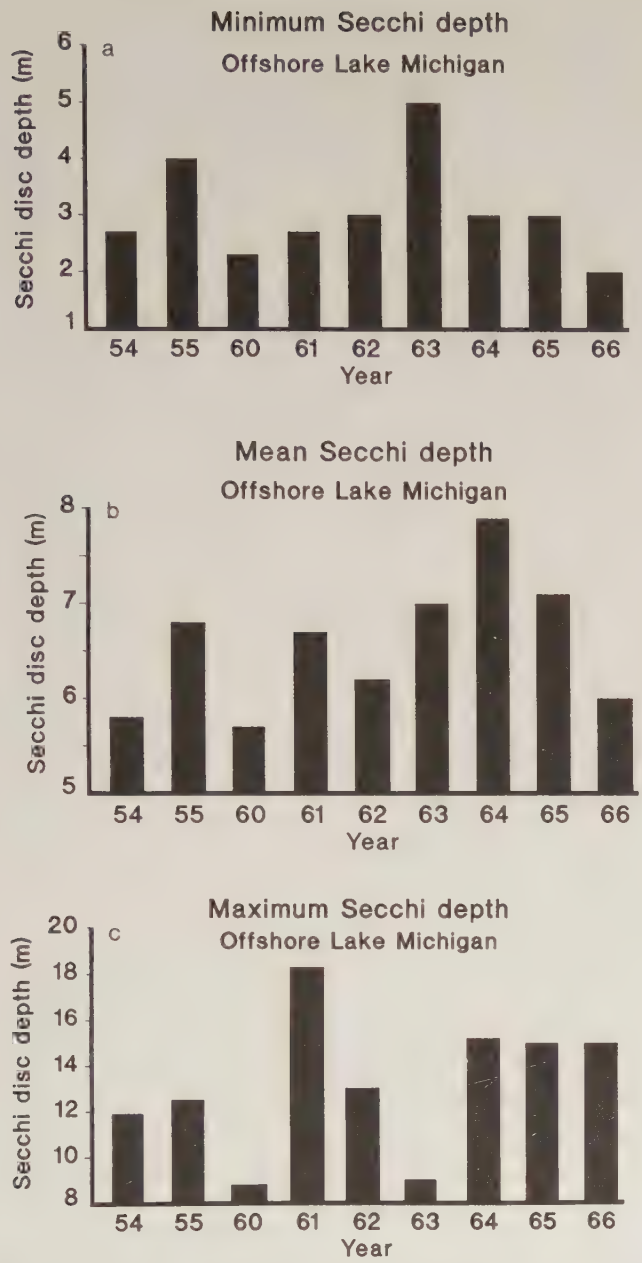

FIG. 5. (a) Mean, (b) minimum, and (c) maximum Secchi disc depths in the offshore region of Lake Michigan for various years of lakewide limnological surveys. Data are from table 1 in Ayers et al. (1967).

75 and 1968–71. Although mean annual phytoplankton abundances were some 51% lower over 1972–75 than 1968–71, most of the decrease was associated with the strong decline in phytoplankton abundances over February–April with a lesser decline over November–December (see Danforth and Ginsburg 1980, fig. 2). As *Daphnia* are exceedingly rare during these periods (Evans et al. 1985), these cladocerans clearly could not have caused decreased algal abundances during early spring and late autumn. Similarly, *Daphnia* must have had a minor (if any) effect on annual trends in phytoplankton abundance as estimated from intake sampling at the Chicago water filtration plant (Fig. 4).

Historic Water Clarity Observations

Like zooplankton, records of phytoplankton abundance in the offshore region are extremely limited prior to the 1970s. However, a number of limnological surveys were conducted in the offshore region during the mid-1950s and 1960s. Secchi disc depth was measured during these surveys and was reported as mean, minimum, and maximum water clarity for the various survey years (Ayers et al. 1967; this study, Fig. 5). These data show no evidence of a decrease in water clarity with the 1954–66 increase in alewife abundances. Mean water clarity was similar in 1954 and 1955 when alewife were rare to that observed in later years when alewife were abundant. Moreover, maximum water clarity (~15 m) was especially high in 1964, 1965, and 1966, exceeding values recorded in 1954 and 1955. Mean Secchi disc depth was significantly ($p = 0.05$) correlated with lakewide alewife abundances ($r = +0.47$) and algal abundances ($r = +0.54$) at the Chicago intake. Maximum Secchi disc depth was similarly significantly correlated with lake alewife abundances ($r = +0.47$) and algal abundances at the Chicago intake ($r = +0.54$). Correlations of minimum Secchi disc depth with annual alewife and algal abundances were not significant. Thus, these Secchi disc data and correlation analyses provide no support for the hypothesis that increased alewife abundances during the early to mid-1960s resulted in a decreased abundance of large-bodied *Daphnia* grazers with a concomitant increase in phytoplankton standing stocks and a decrease in water clarity.

These historic water clarity observations also provide for a further evaluation of the significance of the relatively high Secchi disc depths observed in the summers of 1983 and 1984 (Scavia et al. 1986). High water clarity (~15 m) during these two summers has been attributed to low alewife abundance and intense grazing by a *Daphnia*-dominated zooplankton community (Scavia et al. 1986). However, maximum Secchi disc depths observed in the summer of 1983 and 1984 were similar to those observed during 1964–66, i.e. years of high alewife abundance and low *Daphnia* abundance. Thus, the role of *Daphnia* in reducing phytoplankton standing stocks and improving water clarity is ambiguous not only for the 1950s to the 1970s as discussed by Edgington (1984, 1986) but for the early 1980s (Dorazio et al. 1987; Lehman 1988; Evans 1990; this study).

Conclusions

Some researchers have argued that long-term increases and decreases in Lake Michigan phytoplankton abundance were strongly linked to changes in alewife abundance, i.e. top-down control mechanisms. Such arguments have been based on linking long-term trends in phytoplankton and alewife abundances by invoking a connecting grazing link; the long-term trend on grazing pressure is based on observed differences in zooplankton abundances in the summers of 1954, 1966, and 1968. While these arguments appeared appropriate at the time, continued research into the structure of the Lake Michigan ecosystem has revealed a number of weaknesses in the basic assumptions of the hypothesis. First, the role of alewife in affecting differences in zooplankton abundances in the offshore region of Lake Michigan during the summers of 1954, 1966, and 1968 is not as evident as initially proposed. Therefore, it is inappropriate to infer long-term trends in zooplankton community structure (and grazing) based on our understanding of long-term trends in alewife abundance. Second, estimates of *Daphnia* spp. grazing rates in offshore waters during summer 1954 (and other summers) indicate that such rates were relatively low and unlikely to have had a major effect on phytoplakton abundances unless algal growth rates were exceedingly low. Third, the top-down argument is inappropriately based on linking trends in

phytoplankton abundances in the inshore region with purported trends in zooplankton abundance in the offshore region. Fourth, the top-down argument is based on annual phytoplankton abundance estimates. However, long-term trends in mean annual phytoplankton abundance are strongly weighted by events occurring during early spring and late autumn, periods in which large-bodied *Daphnia* grazers are exceedingly rare. Finally, the discovery of Secchi disc data collected during the 1960s provides no evidence that offshore water clarity diminished with the alewife population explosion. Moreover, maximum water clarity in 1966 was comparable with that observed in summer 1983 when *D. pulicaria* dominated the offshore zooplankton community.

The results of this study do provide some evidence that summer abundances of some zooplankton species in the offshore region of Lake Michigan were significantly correlated with alewife abundances over the 1954–87 period. Such taxa included the large-bodied *E. lacustris*, *D. galeata mendotae*, and *D. sicilis* (negative correlations) and the small-bodied *B. longirostris* (positive correlations); both positive and negative correlations were observed for *L. kindtii*. The magnitude of the correlation depended on the source (location, time period) of the zooplankton and alewife data used in the analyses.

Finally, it is important to note that our understanding of the long-term changes (and causal factors) in the Lake Michigan ecosystem continues to be limited by insufficient data. The three most important limiting factors are insufficient data at the regional level, inconsistent sampling methods and locations, and limited considerations of seasonal effects. In addition, more information is required on ecosystem function including algal growth rates, zooplankton grazing rates, fish predation rates, etc. Future surveillance and monitoring efforts should address these issues.

Acknowledgements

Many agencies provided support to this work including Indiana & Michigan Electric Company, Michigan Sea Grant (grant No. NA-80AA-0-00072), and the U.S. Environmental Protection Agency, Office of Research and Development (grant No. R-812311-01-0). The National Science Foundation (grant No. OCE76-11355 to C. Schelske and R. Moll) provided ship time and some support in 1977, while the NOAA Great Lakes Environmental Research Laboratory (GLERL) provided shiptime in 1982. Dr. Hank Vanderploeg of GLERL collected zooplankton samples in 1984, while Dan Conley of the Center for Great Lakes and Aquatic Studies provided zooplankton samples in 1985. Work on this manuscript was conducted while at the Center for Great Lakes and Aquatic Studies and the National Hydrology Research Institute. Reviewers were Drs. David Jude and Eugene Stoermer of CGLAS and Dr. Hank Vanderploeg. Ms. Ellen Flanagan of the Bureau of Water Operation, Chicago, provided unpublished algal data for the 1978–89 period. Special appreciation is extended to LaRue Wells of the U.S. Fish and Wildlife Service for his many helpful comments on an earlier version of this manuscript and on previous work. Contribution No. 548 CGLAS and CS-92008 NHRI.

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CORRECTIONS TO RECENT PUBLICATIONS
CORRECTIONS SUR RÉCENTES PUBLICATIONS

VOLUME 44, No. 4 — article by R. K. Misra and J. F. Uthe: *Methods of time trends analysis applied to contaminant levels in Canadian Atlantic cod (*Gadus morhua*).*

Page 860, Table 1: The metal concentrations for liver and muscle were inadvertently reversed, i.e. those listed for liver are those for muscle and vice versa. The catch locations, percent fat, and concentrations of PCB, *p,p'*-DDE, and *p,p'*-DDT are all correct. The authors thank Dr. J. Hellou (DFO, St. John's, Newfoundland) for bringing this error to their attention.

ANNOUNCEMENTS/AVIS

International Symposium on Biology and Management of Coregonid Fishes V

1993
Olsztyn, Poland

The Fifth International Coregonid Fishes Symposium will be held 23–26 August 1993 at the University of Agriculture and Technology, Olsztyn, Poland.

The conference topics will include the following:

- Biology, life history, and population dynamics
- Genetics and systematics
- Fisheries and fisheries management
- Impacts of human interventions
- Aquaculture and rearing

The symposium will include special workshops on nomenclature of coregonid forms and populations, computer modelling of coregonid population dynamics, effects of fish introductions, and cage rearing of coregonid larvae.

Papers accepted will be published after the symposium. Abstracts for oral or poster presentation are due **31 December 1992**. For further information, contact Dr. M. Luczynski, Symposium Secretariat, Department of Basic Fishery Sciences, University of Agriculture and Technology, 10-718 Olsztyn-Kortowo Bl. 37, Poland. Telephone: 48 89 278284; Fax: 48 89 278969; Telex: 526419 art pl.

International Symposium on Biology and Management of Coregonid Fishes V

1993
Olsztyn, Pologne

Le cinquième Symposium international sur les corégonidés aura lieu du 23 au 26 août 1993 à l'Université agricole et technique (University of Agriculture and Technology) d'Olsztyn, Pologne.

Ce symposium portera sur les questions suivantes :

- la biologie, le cycle biologique et la dynamique des populations
- la génétique et la systématique
- les pêches et leur gestion
- les impacts des activités humaines
- l'aquiculture et l'élevage

Auront aussi lieu des ateliers de travail spéciaux sur la nomenclature des formes et des populations de corégonidés, la modélisation informatisée de la dynamique des populations de corégonidés, les conséquences de l'introduction de poissons et l'élevage en cages de larves de corégonidés.

Les présentations acceptées seront publiées après le symposium. Les résumés pour la séance d'affichage et les présentations orales doivent être reçus au plus tard **le 31 décembre 1992**. Pour renseignements, contacter la personne suivante : D[r] M. Luczynski, Symposium Secretariat, Department of Basic Fishery Sciences, University of Agriculture and Technology, 10-718 Olsztyn-Kortowo Bl. 37, Poland. Téléphone : 48 89 278284; télécopieur : 48 89 278969; Téléimprimeur : 526419 art pl.

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Subscription Information **ISSN** 0925-1014
1992, Volume 2 (4 issues)
Subscription rate: Dfl.354.00/US$180.00
incl. postage/handling
Special rate for members of the Aquatic Ecosystem Health and Management Society: US$55.00 *incl. postage/handling.*

P.O. Box 322, 3300 AH Dordrecht, The Netherlands
P.O. Box 358, Accord Station, Hingham, MA 02018-0358, U.S.A.

Journal Highlight

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Ministre des Approvisionnements et Services Canada 1992

CJFSDX49(8) 1527–1752 (1992)
ISSN 0706-652X

Subscription rates/Abonnements

Canada: Institutional/Collectif $275; Personal/Personnel $95
Other countries/Autres pays : Institutional/Collectif $330(U.S.); Personal/Personnel $114(U.S.)
Single issue/Par numéro : $16.95 (Canada); $20.35(U.S.) (Other countries/Autres pays)